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ماجســـــــتير العلـــــــوم الحياتيـــــة

التحـاليـــــــــــل الطبيـــــــة

Cystatin C and Some Biochemical Parameters for Dialysis Adequacy Among Hemodialysed Patients at Al-Shifa Hospital, Gaza-Governorate

سيستاتين سي وبعض المعايير البيوكيميائية لقياس كفاءة الغسيل الدموي لدى مرضى الفشل الكلوي المزمن في مستشفى الشفاء – محافظة غزة

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إقــــــرار

أنا الموقع أدناه مقدم الرسالة التي تحمل العنوان:

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I

نتيجة الحكم على أطروحة ماجستير

بناءً على موافقة عمادة البحث العلمي والدراسات العليا بالجامعة الإسلامية بغزة على تشكيل لجنة الحكم على أطروحة الباحث/ **فواز حسن محمد العجلة** لنيل درجة الماجستير في كلية **العلوم**/ برنامج **العلوم الحياتية/تحاليل طبية** وموضوعها:

سيستاتين سي وبعض المعايير البيوكيميائية لقياس كفاءة الغسيل الدموي لدى مرضى الفشل الكلوي المزمن في مستشفى الشفاء محافظة غزة

Cystatin C and Some Biochemical Parameters for Dialysis Adequacy Among Hemodialysed Patients at Al-Shifa Hospital, Gaza-Governorate

وبعد المناقشة التي تمت اليوم الاثنين 25 صفر 1440هـ الموافق 2018/11/05م الساعة الثامنة والنصف صباحاً، في قاعة مبنى كلية اجتمعت لجنة الحكم على الأطروحة والمكونة من:

أ. د. بكر محمود الزعبوط	مشرفاً ورئيساً	....
أ. د. يوسف ابراهيم الجيش	مناقشاً داخلياً	....
د. أيمن مصطفى أبو مصطفى	مناقشاً خارجياً	....

وبعد المداولة أوصت اللجنة بمنح الباحث درجة الماجستير في كلية العلوم/برنامج العلوم الحياتية/تحاليل طبية.

واللجنة إذ تمنحه هذه الدرجة فإنها توصيه بتقوى الله تعالى ولزوم طاعته وأن يسخر علمه في خدمة دينه ووطنه.

والله ولي التوفيق،،،

عميد البحث العلمي والدراسات العليا

أ. د. مازن إسماعيل هنية

اللغة	ع	الرقم العام للنسخة	3/5/7191

الموضوع/ استلام النسخة الإلكترونية لرسالة علمية

قامت إدارة المكتبات بالجامعة الإسلامية باستلام <u>النسخة الإلكترونية</u> من رسالة

الطالب/ سلطان محمد حمد العربي

رقم جامعي: 120130593 قسم: التحاليل الطبية كلية: العلوم الحياتية

وتم الاطلاع عليها، ومطابقتها بالنسخة الورقية للرسالة نفسها، ضمن المحددات المبينة أدناه:

- تم إجراء جميع التعديلات التي طلبتها لجنة المناقشة.
- تم توقيع المشرف/المشرفين على النسخة الورقية لاعتمادها كنسخة معدلة ونهائية.
- تم وضع ختم "عمادة الدراسات العليا" على النسخة الورقية لاعتماد توقيع المشرف/المشرفين.
- وجود جميع فصول الرسالة مجمَّعة في ملف (WORD) وآخر (PDF).
- وجود فهرس الرسالة، والملخصين باللغتين العربية والإنجليزية بملفات منفصلة (WORD+ PDF)
- تطابق النص في كل صفحة ورقية مع النص في كل صفحة تقابلها في الصفحات الإلكترونية.
- تطابق التنسيق في جميع الصفحات (نوع وحجم الخط) بين النسخة الورقية والإلكترونية.

<u>**ملاحظة: ستقوم إدارة المكتبات بنشر هذه الرسالة كاملة بصيغة (PDF) على موقع المكتبة الإلكتروني.**</u>

والله وليّ التوفيق،

إدارة المكتبة المركزية

توقيع الطالب

Abstract (English)

Background: Chronic kidney disease (CKD) is a global public health problem with increasing incidence, prevalence, high morbidity and mortality. CKD is a progressive and irreversible loss of renal function and finally complete loss of kidney function or kidney damage, that is, end-stage renal disease (ESRD). Cystatin C (Cys-C) correlates with direct measures of glomerular filtration rate (GFR) more precisely than creatinine.

Objective: To evaluate Cys-C and some biochemical parameters for dialysis adequacy among hemodialysed patients at Al-Shifa hospital, Gaza governorate.

Materials and Methods: This study design was observational cross section pre and post design. It was carried out in Gaza Governorate. A total of 80 CKD with ESRD patients, on regular twice or three-weekly 4-hour hemodialysis, divided into 40 males and 40 females. Anthropometric evaluation and biochemical detection were carried out for kidney function, total protein, albumin, phosphorus, electrolytes, glucose, lipid profile and Cys-C were measured in a single treatment at pre and post dialysis. The data were analyzed using SPSS version 22.

Results: All of the 80 patients were kidney dialyzed in 32 dialysis machines. About 66 (82.5%) were used less than 5 years whereas the rest number were old machine 14 (17.5%). The result showed that there was a highly decrease significant in body mass index (BMI) and weight among the study population (P=0.000). Moreover, a highly decrease significant in urea, creatinine, uric acid and Cys-C were observed (P=0.000). In addition, a highly increase significant level in glucose, lipid profile, total protein and albumin were observed (P=0.000). Furthermore, a highly increase significant in blood Na^+, tCa^+ and iCa^{++} while decrease in K^+, Cl^- and phosphorus levels were observed (P=0.000). In the other hands, the results showed that there was no significant relation between Cys-C reduction ratio (CCRR) with urea reduction ratio (URR), and creatinine reduction ratio (CrRR). Furthermore, the results showed that there was significant correlation between the URR with, Kt/V, SP-Kt/V, CrRR and CCRR (r>65.0%, P<0.05). Moreover, there were no significant differences between Kt/V and SP-Kt/V among study population (P=0.006), that have the same adequacy dose of dialysis. In addition, our findings showed that the percent adequacy cutoff value for CrRR was 62.75% and 24.03% for CCRR

Conclusions: Hemodialysis have highly decrease significant effect on BMI, weight and blood pressure, urea, creatinine, uric acid and Cys-C levels among of study population. The estimated adequacy cutoff value was 62.75% for CrRR and 24.03% for CCRR. Furthermore, the Kt/V and SP-Kt/V they showed the same adequacy dose of dialysis.

Keywords: Hemodialysis adequacy, chronic kidney disease, cystatin C, Kt/v and single pool Kt/V.

ملخص الدراسة (عربي)

مقدمة: تُعَرَّف أمراض الكلي المزمنة بأنها فقدان تدريجي ونهائي لوظائف الكلى ينتهي بتلفها فيما يعرف بالمرحلة النهائية من مرض الفشل الكلوي وتعتبر مشكلة صحية عالمية ذات زيادة مطردة في معدلات حدوثها وانتشارها والوفيات بسببها، بزيادة حدوثه وانتشاره وارتفاع معدلات الاعتلال والوفيات. كما يُعتبر السيستاتين سي أحد المؤشرات البيولوجية الجيدة لوظائف الكلى حيث يُشير بشكل أكثر دقة إلى معدل الترشيح الكلوي من الكرياتينين.

الهدف: تهدف الدراسة لتقييم السيستاتين سي وبعض المعايير البيوكيميائية كمؤشرات لقياس كفاءة الغسيل الدموي لدى مرضى الفشل الكلوي المزمن في مستشفى الشفاء ـ محافظة غزة

الطرق والأدوات: تعتبر هذه الدراسة تحليلية (قبل وبعد)، حيث أجريت الدراسة في محافظة غزة، على مجموعة 80 مريض (40 ذكور، 40 اناث) مصابين بالفشل الكلوي اختيروا عشوائياً، حيث كانوا على جدول علاجي منتظم للغسيل الدموي مرتين او ثلاث مرات أسبوعيا، لمدة أربع ساعات لكل جلسة. وقد تم تقدير المقاييس الجسمانية وقياس التراكيز البيوكيميائية في السيرم لكل من فحوصات وظائف الكلى والبروتين الكلي والألبيومين وأملاح الجسم والدهون والسكر والسيستاتين سي وتم القياس لمرة واحدة خلال الجلسة قبل وبعد الغسيل. تم تحليل البيانات المجموعة باستخدام برنامج الحزمة الإحصائية للعلوم الاجتماعية (SPSS) النسخة 22.

النتائج: شملت الدراسة 80 مريض مصابين بالفشل الكلوي المزمن كانوا يستخدموا 32 جهاز لغسيل الدم، حوالي 66 (82.5 %) منهم كانوا على أجهزة حديثة فترة استخدامها أقل من خمس سنوات، بينما العدد المتبقي 14 كانوا على أجهزة قديمة 14(17.5%). أظهرت النتائج انخفاضا كبيراً ذو دلالة إحصائية في مؤشر كتلة الجسم ووزن الجسم لدى عينة الدراسة (P=0.000) . كما سجلت الدراسة أيضاً انخفاض كبير ذو دلالة إحصائية في مستويات كل من اليوريا والكرياتينين وحمض اليوريك والسيستاتين سي (P=0.000) . بالإضافة انه، لوحظ زيادة ذات دلالة إحصائية في مستويات السكر والكولسترول والدهون الثلاثية والدهون عالية الكثافة والدهون منخفضة الكثافة والبروتين والألبومين في السيرم(P=0.000) . علاوة على ذلك، لوحظ زيادة ذات دلالة إحصائية في مستويات الصوديوم والكالسيوم المرتبط والكالسيوم الغير مرتبط، بينما لوحظ انخفاض في مستويات البوتاسيوم والكلوريد والفوسفور(P=0.000) .ومن ناحية أخرى، أظهرت النتائج عدم وجود علاقة إحصائية بين نسبة كل من CCRR مع CrRR و URR . ايضاً سجلت الدراسة وجود ارتباط قوي وذو دلالة إحصائية بين URR وكل من CrRR و Kt/V و SP-Kt/V وCCRR (r>65.0%, P<0.05). أظهر النتائج تساوي نسب مؤشر الكفاءة لكل من (Kt/V) و(SP-Kt/V) بين أفراد عينات الدراسة (P=0.006). بالإضافة الى ذلك، أظهرت النتائج أن القيمة الحدية لنسبة CrRR كانت (62.75%) ولنسبة CCRR كانت (24.03%).

الاستنتاجات: الغسيل الدموي لمرضى الفشل الكلوي المزمن له تأثير خافض على كل من مؤشر كتلة الجسم، والوزن وضغط الدم ومستويات اليوريا والكرياتينين وحمض اليوريك والسيستاتين سي. وقُدرت نسبة القيمة الحدية لعينات الدراسة لـ CrRR ب 62.75٪. حيث بلغت الـ 24.03% في حالة CCRR. علاوة على ذلك، أظهرت النتائج ان Kt / V و SP- Kt/V تساويهما في القيمة.

الكلمات المفتاحية: كفاءة الغسيل الدموي، مرض الكلى المزمن، سيستاتين سي، Kt /V و Single pool- Kt/V.

Dedication

With all the love and heartfelt gratitude these simple words can

convey, to all of them I dedicate this work:

To my father and my mother who taught me how to give

To my lovely wife who supported me wholeheartedly

To my children (Bahaa, Deyaa, Alaa, Hassan,

Dema, layan and Dana)

To my brothers and sisters

To all my friends who spare no effort to help me

To all my teachers who supported me

To all my colleagues who supported me

This work is also dedicated to:

to all the Palestinian martyrs and people who have suffered and struggled to

have a free Palestine.

Acknowledgements

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ACR:	Albumin/Creatinine Ratio
AKI:	Acute Kidney Injury
ARF:	Acute Renal Failure
ATN:	Acute Tubular Necrosis
AV:	Arteriovenous
AVF:	Arteriovenous Fistula
BMI:	Body Mass Index
BUN:	Blood Urea Nitrogen
CBC:	Complete Blood Count
CCRR:	Cystatin C Reduction Ratio
CG:	Cockcroft-Gault
CKD:	Chronic Kidney Disease
Cl^-:	Chloride
CrCl:	Creatinine Clearance
CRF:	Chronic Renal Failure
CrRR:	Creatinine Reduction Ratio
CVD:	Cardiovascular Disease
Cys-C:	Cystatin C
DBP:	Diastolic Blood Pressure
eGFR:	Estimate Glomerular Filtration Rate
ESRD:	End-Stage Renal Disease
FF:	Filtration Fraction
FIA:	Fluorescence Immunoassay
GBM:	glomerular basement membrane
GFR:	Glomerular Filtration Rate
HD:	Hemodialysis
HDL:	High-Density Lipoprotein
HF:	High- Flux
HIV:	Human Immunodeficiency Virus
$i.Ca^{++}$:	Ionized Calcium
ISE	Ion Selective Electrode
K^+:	Potassium
Kt/v:	Treatment Index formula
LDL:	Low Density Lipoprotein
LF:	Low- Flux
MDRD:	Modification Of Diet In Renal Disease
Mg^{++}:	Magnesium
MM:	Middle Molecules

MW:	Molecular Weight
Na$^+$:	Sodium
NCDS:	National Cooperative Dialysis Study
NKF:	National Kidney Foundation
NKF-KDOQI:	National Kidney Foundation -Kidney Dialysis Outcomes Quality Initiative
PCR:	Protein/Creatinine Ratio
PD:	Peritoneal Dialysis
PENIA:	Particle Enhanced Nephelometric Immunoassay
PETIA:	Particle Enhanced Turbidimetric Immunoassay
Pmp:	Per Million Population
RRT:	Renal Replacement Therapy
SBP:	Systolic Blood Pressure
SLE:	Systemic Lupus Erythematosus
Sp-Kt/V:	Single-Pool Kt/V
t.Ca$^+$:	Total Calcium
UF:	Ultrafiltration
UKM:	Urea Kinetic Modeling
URR:	Urea Reduction Ratio

Chapter -1
Introduction

Chapter -1
Introduction

1.1 Overview

Chronic kidney disease (CKD) has been recognized as a global public health problem with increasing incidence, prevalence, high morbidity and mortality (**Levey et al., 2007; and Tsai et al., 2010**). CKD is defined as a reduced glomerular filtration rate, increased urinary albumin excretion, or both, and is an increasing public health issue. Prevalence is estimated to be 8–16% worldwide (**Jha et al., 2013**). CKD is a progressive and irreversible loss of renal function. The appearance of proteinuria and elevated serum creatinine, representing a decrease in the GFR, and finally complete loss of kidney function or kidney damage, that is, end-stage renal disease (ESRD) (**Bakris et al., 2000**). CKD is determined either directly by biopsy, or indirectly by the presence of proteinuria, microalbuminuria, abnormal urinary sediment or abnormal findings in imaging studies or by a GFR <60ml/min/1.73m^2 for at least three months (**Levey et al., 2003**).

CKD considers a developing process that is initiated by different causes, all with the common end result and usually progressive damage of varying severity to the kidney. These patients have a continuous decline in renal function and hence are said to have progressive renal failure (**Vijayakumar et al., 2007**). As kidney disease advances and the GFR declines, almost all of the body's systems are adversely affected. The major complications of CKD are cardiovascular disease (CVD), renal Osteodystrophy, anemia, and nutritional disturbances (**Cassidy et al., 2007**).

The major risk factors for CKD include older age, sex family history of CKD, diabetes mellitus, hypertension, CVD, metabolic syndrome, and obesity (**Taal and Brenner, 2007**). blood pressure changes, fluid imbalance, anemia, calcium/phosphorus metabolism, hypoalbuminemia, hyperhomocysteinemia, malnutrition, inflammation, oxidant stress, insulin resistance, altered renin angiotensin axis and endothelial dysfunction (**Amaresan, 2005**).

Chronic renal failure (CRF) occurs once a kidney is damaged and cannot work effectively. Kidneys remove waste from the blood, which passes out of the body in urine. If the disease is diagnosed early, the kidney damage can be slowed, but not

stopped completely. CRF is often a result of diseases such as diabetes, high blood pressure, and other kidney diseases(kidney stone, benign prostatic hypertrophy, polycystic kidney disease, drug-induced kidney disease). In few patients, severe infections (eg, hepatitis B or human immunodeficiency virus (HIV) or autoimmune diseases (e.g. lupus) can also cause kidney disease **(Dirks et al., 2006).**

The assessment of GFR is essential for clinical practice it is crucial for deciphering the symptoms, signs, and laboratory abnormalities that might signify kidney disease, for drug dosing, and for detecting, managing and estimating the prognosis of CKD **(Stevens et al., 2006).** GFR provides an excellent measure of the filtering capacity of the kidneys. A low or decreasing GFR is a good index of kidney disease **(National Kidney Foundation, 2002).** The ideal marker of GFR should be an endogenous molecule, which being produced at a constant rate, is cleared solely by the kidneys via free glomerular filtration, with being neither secreted by tubular cells nor reabsorbed into peritubular circulation **(Sirwal et al., 2004).** Despite advances in GFR measurement since then, it remains a specialized diagnostic test. In routine clinical practice, GFR is estimated from the serum concentrations of endogenous filtration markers **(Steindel et al., 2000.; and Levey et al., 2010).**

The first widely used GFR equations to estimate creatinine clearance in adults from serum creatinine were developed in the 1970s. In recent years, a number of new equations have been advanced for use with standardized serum creatinine assays and gained worldwide acceptance for implementation into clinical practice as a "first test" for assessing GFR in adults. Recently, equations using standardized Cys-C assays have been proposed as a "confirmatory test" for decreased estimate GFR from creatinine **(Levey et al., 2009.; Inker et al., 2011.; and Inker et al., 2012).**

Cys-C is a protein inhibitor of cysteine proteinases that is synthesized at a stable rate by all nucleated cells. Because of its low molecular weight and high isoelectric point, it can be eliminated almost exclusively by glomerular filtration **(Grubb, 2001).** Cys-C concentration is not influenced by age, sex, or protein ingestion, and it is sensitive to small changes in glomerular filtration **(Filler et al., 2005).** Because of these characteristics Cys-C concentration is considered among the best markers of glomerular filtration status **(Acuña et al., 2009).**

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The blood plasma proteins with molecular masses below 15-25 kDa are generally almost freely filtered through the normal glomerular membrane and then almost completely reabsorbed and degraded by the normal proximal tubular cells. Consequently, also the molecular mass of Cys-C is 13 kDa and with a probable ellipsoid shape with axes of about 30 and 45 A° (**Bode et al., 1988**). Indeed, studies of the handling of human Cys-C in the rat have shown that the plasma renal clearance of Cys-C is 94% of that of the generally used GFR marker [51Cr-EDTA] and that Cys-C thus is practically freely filtered in the glomeruli (**Tenstad et al., 1996**). At least 99 % of the filtered Cys-C was found to be degraded in the tubular cells.

Cys-C is a 122- amino acid, it is a member of a family of competitive inhibitors of cysteine proteases (**Mussap and Plebani, 2004**). The human Cys-C gene is of the housekeeping type, which indicates a stable production rate of Cys-C by most nucleated cell types. Cys-C has numerous properties that make it a good marker of GFR, including a constant production rate, free filtration at the glomerulus, complete reabsorption, and catabolism by the proximal tubules without reabsorption into the bloodstream, and no renal tubular secretion (**Levin, 2005**). Serum Cys-C is a good marker of renal function and correlates well to direct measures of GFR more precisely than creatinine, because its serum concentrations are independent of muscle mass and do not seem to be affected by age or sex (**Dharnidharka et al., 2002.; and Perkins et al., 2005**). The development of automated and rapid particle-enhanced immunoturbidimetric and immunonephelometric methods have permitted large-scale use of serum Cys-C as a clinically useful GFR marker. However, different factors have been reported to affect the production of Cys-C; huge doses of glucocorticoids have been described to increase the production of Cys-C, whereas low and medium doses of glucocorticoids do not seem to alter the production of Cys-C (**Bokenkamp et al., 2002**).

Thyroid dysfunction also has a major impact on Cys-C level. In contrast to creatinine concentrations, Cys-C levels are lower in the hypothyroid and higher in the hyperthyroid state as compared with the euthyroid state (**Fricker et al., 2003.; Knight et al., 2004.; and Mussap and Plebani, 2004**). Therefore, as Cys-C is a more accurate surrogate marker of renal function compared with serum creatinine,

the level of serum creatinine has been used to assess renal function but is often affected by muscle mass, which is dependent on age, weight, and gender (**Levey et al., 1988**). Serum Cys-C was recently proposed as an alternative marker of GFR, and its higher performance compared with creatinine has been suggested from a meta-analysis (**Dharnidharka et al., 2002**). Dialysis modality selection for ESRD patients, there are three primary treatment options, Hemodialysis (HD), Peritoneal dialysis (PD), and kidney transplantation (**United States Renal Data System, USRDS, Annual Data Report, 2009**). In renal failure, filtrate formation decreases or stops completely. Because toxic wastes accumulate rapidly in the blood when the kidney tubule cells are not functioning, dialysis by an artificial kidney is necessary to cleanse the blood while the kidneys are shut down (**Elaine, 2003**).

HD is a medical procedure that uses a specific machine (a dialysis machine) to filter waste products from the blood and to return normal constituents to it. HD is frequently done to treat ESRD. Under such circumstances, kidney dialysis is typically administered by a fixed schedule of three times per week. (**Crawford and Lerma, 2008**). The basic principle of the artificial kidney is to pass blood through minute blood channels bounded by a thin membrane. On the other side of the membrane is a dialyzing fluid into which undesirable substances in the blood pass by diffusion. (**Guyton and Hall, 2011**). The majority of patients with CRF, between 9 and 12 hours of dialysis is weekly required, usually divided into three equal sessions. The removal of free water during HD is called ultrafiltration. It occurs when water driven by either a hydrostatic or osmotic force is pushed through the membrane of the dialyzer (**Nanovic, 2005**). However, the dialysis dose must be individualized. Lately there has been much concern in the possibility that more frequent dialysis may be associated with enhanced outcomes in Patients (**Dennis et al., 2005**).

Dialysis clearance is defined as the volume of blood from which all solutes in question is reduced during a specified time period. The total amount of solute removal depends on the duration of the therapy (**Shih-Han, 2013**). The duration and frequency of HD treatments can also affect a patient's total solute clearance, although conventional HD three times per week, four hours per session It has highly efficient in removing small solutes, and highly clearance rate per session (**Gotch. 1998 and FHN Trial Group et al., 2010**). Dialysis adequacy is equalizing the

kidneys function results of patients undergoing HD as those of healthy people, It's the amount of dialysis required to keep a patient alive and relatively asymptomatic **(Mehta et al., 2010 and Biniaz et al., 2018).** It refers to delivery of a treatment dose that is considered sufficient to promote optimal long-term outcome, it is assessed by the removal of urea, calculated and expressed either by the URR CrRR and CCRR or by the treatment index Kt/V and Sp-Kt/V **(Maheshwari et al., 2016 and Johnson et al., 2015**).

The quality of HD is associated with the patient's general health, fewer complications of renal failure, and consequently, higher life span **(Biniaz et al., 2018).** Insufficient HD is one of the most important causes of morbidity and mortality in HD patients **(Nemati, 2017).** If Organic Uremic compounds removal is inadequate, then dialysis is inadequate, regardless of the serum concentration level for each compound **(Maheshwari et al., 2016 and Johnson et al., 2015**).
The insufficient dose of HD increases the duration of hospitalization and costs imposed on the patients. The efficiency of HD is a significant index which estimates sufficient doses of dialysis for the patients with ESRD **(Nemati, 2017).**

Urea kinetic modeling (UKM) is currently the preferred method for determining Kt/V by the National Kidney Foundation KDOQI Guidelines. Several different UKMs have been developed to quantify Kt/V, including the single pool Kt/V, equilibrated Kt/V, and weekly standard Kt/V **(Kdoqi, 2006**). Kt/V is one of the most common methods to calculate HD effectiveness. it includes the clearance of urea and duration of dialysis and distribution volume of urea in the body. This method is used from past times in researchers as an indicator of dialysis efficiency and its association with the rate of mortality and complications **(Hakim, 1990 and Nemati, 2017).**

Recent methods for assessment of dialysis dose are based on the pre-dialysis and post-dialysis difference in serum urea, creatinine, Cys-C and include URR, CrRR and Cys-CRR, sp-Kt/V, equilibrated Kt/V (e-Kt/V), and weekly standard Kt/V (std-Kt/V) **(Levin, 2006.; Johnson et al., 2015 and Maheshwari et al., 2016**).

1.2 Objectives:

1.2.1 General objective:

The overall aim of the present study was evaluated cystatin C and some biochemical parameters for dialysis adequacy among hemodialysed patients at Al-Shifa hospital, Gaza- governorate.

1.2.2 Specific objectives:

- To determine serum Cys-C and other biochemical parameters including urea, creatinine, uric acid, BUN, total protein, albumin, phosphorus, and serum electrolytes (Na^+, K^+, tCa^+, iCa^{++} and Cl^-) and cholesterol, triglycerides, high-density lipoprotein (HDL-C) and low density lipoprotein (LDL-C) in hemodialysed patients.

- To assess pre and post serum Cys-C for dialysis adequacy among hemodialysed Patients.

- To test the correlation CCRR with URR, Kt/V, SP-Kt/V and CrRR.

- To assess of dialysis dose and the removal of uremic toxins, urea, creatinine and Cys-C by calculating URR, CrRR and CCRR, and the treatment index by Kt/V and Sp-Kt/V.

- To estimate of the cut-off value for CCRR and CrRR.

- To compare of hemodialysis adequacy markers URR, CrRR, CCRR, Kt/V and SP-Kt/V with clearance standers based on adequacy.

1.3 Significance of the study:

- Chronic kidney disease among hemodialysis patients is a major global public health issue. Rates are expected to increase, largely due to the growth of diabetes, cardiovascular and hypertension.

- In the Gaza Strip only one study evaluated serum levels of cystatin C and other markers of DNP among a group of type 2 diabetes mellitus patients in early stage renal disease **(Raffat, 2013).**

- This is the first study to assess serum Cys-C, urea and creatinine as biomarker to estimate dialysis adequacy and to determine of dialysis dose and the removal of

uremic toxins, urea, creatinine and Cys-C by calculating URR, CrRR and CCRR, and the treatment index Kt/V and Sp-Kt/V among CKD hemodialysed patients in Gaza Strip.

− The usefulness of serum Cys-C as an indicator of the dialysis adequacy is not known with certainty. Therefore, the present study tries to evaluate the potential clinical significance of serum Cys-C as a hemodialysis adequacy marker and compared to other adequacy markers (Urea, Creatinine, Kt/V and SP-Kt/V).

Chapter -2
Literature Review

Chapter -2

Literature review

2.1 The kidneys

2.1.1 Location and structure

Most humans have two kidneys, which are located in the retroperitoneal space on each side of the abdominal aorta **(Briggs et al., 2014)**, against the dorsal body wall beneath the parietal peritoneum in superior lumbar region where they receive some protection from the lower part of the rib cage.

The right kidney is positioned slightly lower than the left kidney **(Marieb, 2003)**, The weight of each kidney ranges from 125 g to 170 g in the adult male and from 115 g to 155 g in the adult female. The human kidney is approximately 11 cm to 12 cm in length, 5.0 cm to 7.5 cm in width, and 2.5 cm to 3.0 cm in thickness **(Luyckx and Brenner, 2005)**. It is bean-shaped and contains approximately 400,000 to 800,000 nephrons in the renal cortex **(Briggs et al., 2014)**.

Each kidney has a medial indentation (the hilus) in which there is two renal arteries, renal vein, and ureter. A fibrous renal capsule encloses each kidney. The kidney has three regions, outer granulated layer called renal cortex, renal medulla that consists of cone shaped tissue masses called medullary pyramids, and renal pelvis which is a central space or cavity that is continuous with the ureter **(Figure: 2.1) (Marieb, 2003).**

Figure (2.1): Location and gross anatomy structure of the kidney.

(a) Anterior view of organs of The urinary system. **(b)** Position of the kidneys against the posterior body wall, cross-section viewed from inferior direction. **(c)** Posterior in situ view showing the relationship of the kidneys to the 12th rib pair. **(d)** A sagittal section of the kidney showing the blood supply. **(e)** The same section showing the renal cortex, the renal medulla, and the renal pelvis, which connects with the ureter. **(f)** An enlargement showing the placement of nephrons **(Marieb, 2003.; and Mader, 2004).**

The functional unit of the kidney is the nephron. Each kidney contains approximately one million tiny structures called nephrons **(Figure: 2.1).** Nephrons are responsible for the processes of filtration, reabsorption, and secretion that go on in the kidney to form the urine product. The nephron consists of two main structures, a glomerulus, which is a knot of capillaries, and a renal tubule. The closed end of the renal tubule is enlarged and cup-shaped and completely surrounds the glomerulus. This portion of the renal tubule is called Bowman's capsule. In order from Bowman's capsule they are the proximal convoluted tubule, loop of Henle, and the distal convoluted tubule. Most of the nephron is located in the cortex, only portion of the loops of Henle dip into the medulla. Urine from many nephrons is collected in the collecting ducts, which deliver the final urine product into the calyces and pelvis of the kidney **(Thibodeau and Patton, 2013).**

This is further emptied into the ureter through peristalsis initiated by special pacemaker cells and squirted into the bladder as urine. Once a certain bladder pressure is reached, the urine is voided through the urethra **(Koeppen and Stanton, 2012).** Every nephron is associated with two capillary beds: The glomerulus and the peritubular capillary bed. The glomerulus is both fed and drained by arterioles. The afferent arteriole is the feeder vessel, and the efferent arteriole receives blood that has passed through the glomerulus. The efferent arteriole then breaks up to form the peritubular capillary bed, which closely clings to the whole length of the tubule. The peritubular capillaries then drain into an interlobular vein that leaves the cortex **(Marieb, 2003).**

Figure (2.2): Anatomy and Structure of the nephron. A nephron is made up of a glomerular capsule, the proximal convoluted tubule, the loop of the nephron, the distal convoluted tubule, and the collecting duct **(Marieb, 2003.; and Mader, 2004).**

2.1.2 Role of the kidneys

The kidneys perform two main functions: eliminate soluble waste products of metabolism and preserve the internal environment of the cells (maintain water balance, pH, ionic equilibrium, and fluid osmotic pressure) **(Kaplan and Szabo, 1983).** The kidneys extract waste from blood, balance body fluids, form urine, and aid in other important functions of the body. the kidneys filter waste products from the blood before converting them into the urine **(Chand, 2015).** The kidneys also help maintain blood pressure, maintain the correct levels of chemicals in your body which, in turn, will help heart and muscles function properly, produce the active form of vitamin D (1, 25-dihydroxycholecalciferol) that keeps bones healthy, produce a substance called erythropoietin, which stimulates production of red blood cells **(Chand, 2015).**

2.1.3 Formation of urine

Urine formation starts with the ultrafiltration of blood in the kidney glomerulus. Plasma flows through the pores in the endothelium, while blood cells are retained in the capillary lumen. The glomerular basement membrane (GBM) is thought to act as a prefilter, which prevents proteins from passing through the capillary wall. Finally, the filtrate is guided through a slit between two podocyte cell protrusions to the Bowman.s space and further to the tubular system of the nephron, where it is concentrated to become the final urine **(Vesa, 2004).**

2.1.4 Abnormal urinary constituents

Table (2.1): Abnormal urinary constituents

Substance	Name Of Condition	Possible Causes
Glucose	Glycosuria	Diabetes mellitus
Proteins	Proteinuria, or albuminuria	Nonpathological: excessive physical exertion, pregnancy, high-protein diet Pathological (over 250 mg/day): heart failure, severe hypertension, glomerulonephritis, often initial sign of asymptomatic renal disease
Ketone bodies	Ketonuria	Excessive formation and accumulation of ketone bodies, as in starvation and untreated diabetes mellitus
Hemoglobin	Hemoglobinuria	Various: transfusion reaction, hemolytic anemia, severe burns, etc.
Bile pigments	Bilirubinuria	Liver disease (hepatitis, cirrhosis) or obstruction of bile ducts from liver or galbladder
Erythrocytes	Hematuria	Bleeding urinary tract (due to trauma, kidney stones, infection, or neoplasm)
Leukocytes (pus)	Pyuria	Urinary tract infection

Adopted from (Marieb, 2003).

2.1.5 Principles of renal pathophysiology

Renal injury can be characterized as either acute or chronic. Each has a distinctive clinical expression.

2.1.5.1 Acute renal failure

Acute renal failure (ARF) also called acute kidney failure or acute kidney injury is characterized by a rapid decline in GFR over hours to days **(Jameson, 2010).** This condition is usually marked by a rise in serum creatinine concentration or azotemia (a rise in blood urea nitrogen concentration) immediately after a kidney injury. Emergency dialysis may be needed until the situation resolves and the kidneys begin functioning again **(Agraharkar, 2007).**

Retention of nitrogenous waste products, oliguria (urine output <400 mL/d contributing to extracellular fluid overload), and electrolyte and acid-base abnormalities are frequent clinical features. ARF is usually asymptomatic and diagnosed when biochemical monitoring of hospitalized patients reveals a new increase in blood urea and serum creatinine concentrations **(Jameson, 2010).** Acute renal insufficiency typically presents with the symptoms of volume overload secondary to impaired urine formation or excretion. The consequent retention of

sodium and therefore of water can cause an expansion of the intravascular spaces and extravasation of fluid into the interstitial space throughout the body. The resulting volume expansion can therefore present as peripheral edema, pulmonary edema, or congestive heart failure. In acute renal failure, both acidemia (resulting from failure to excrete or buffer the endogenous metabolic production of acids) and hyperkalemia (resulting from the lack of excretion of dietary potassium) can result in cardiac arrhythmias and sudden death. Acute uremia has a particularly inhibitory effect on platelet function resulting in an increase in the bleeding tendency **(Schreiner and Kissane, 1990).**

2.1.5.2 Types of acute renal failure:

ARF is generally divided into three major categories: (1) diseases that cause renal hypoperfusion, resulting in decreased function without frank parenchymal damage (prerenal ARF, or azotemia) (∼55%); (2) diseases that directly involve the renal parenchyma (intrinsic ARF) (∼40%); and (3) diseases associated with urinary tract obstruction (postrenal ARF) (∼5%) **(Jameson, 2010).** Acute renal failure may be pre-renal, renal or post-renal **(Figure: 2.3)**.

Figure (2.3): Different types of acute renal failure

2.1.5.2.1 Pre-renal failure

It is a Combination of hypotension, hypovolemia resulting in diminished renal perfusion is the most common cause of ARF in hospitalized patients, in pre-renal failure, the renal tissue is intact and kidney biopsy shows normal renal histology? Oliguria and high serum creatinine are due to functional impairment; since there is no sufficient blood reaching the kidney to be cleared of these toxins. Other causes of

pre-renal failure- not necessarily associated with a decrease in GFR- are conditions that increase urea production such as large protein intake and increased protein catabolism (fever, surgery, severe illness, steroids and tetracycline).

2.1.5.2.2 Intrinsic renal failure

there is a damage involving the glomeruli, renal tubules or tubulointerstitium with loss of their functions. Consequently, wastes accumulate with increase in serum urea and creatinine. Intrinsic renal failure includes acute glomerulonephritis, acute interstitial nephritis and acute tubular necrosis (ATN).

2.1.5.2.3 Post-renal failure

It is ARF from obstruction to urine flow between the external urethral meatus and bladder neck, bilateral ureteric obstruction, or unilateral ureteric obstruction in a patient with one functioning kidney or with significant preexisting CKD. Bladder neck obstruction is the most common cause of post-renal ARF and is usually due to prostatic disease (e.g., hypertrophy, neoplasia, or infection), neurogenic bladder, or therapy with anticholinergic drugs **(Jameson, 2010).** The obstruction of the urinary tract results in increasing the pressure above the level of the obstruction up to the nephron including the urinary space of the renal glomeruli. When this back pressure exceeds that of the filtration pressure in the renal glomeruli, the process of urine formation will stop with progressive accumulation of wastes and increase of serum creatinine and blood urea **(Sobh, 2000).**

Less common causes of acute lower urinary tract obstruction include blood clots, calculi, and urethritis with spasm. Ureteric obstruction may result from intraluminal obstruction (e.g., calculi, blood clots, sloughed renal papillae), infiltration of the ureteric wall (e.g., neoplasia), or external compression e.g., retroperitoneal fibrosis, neoplasia or abscess, inadvertent surgical ligature **(Jameson, 2010).**

2.1.5.3 Chronic renal failure

The term chronic renal failure (CRF) applies to the process of continuing significant, irreversible reduction in nephron number, and typically corresponds to CKD stages 3–5 **(Jameson, 2010).** The dispiriting term ESRD represents a stage of

CKD where the accumulation of toxins, fluid, and electrolytes normally excreted by the kidneys results in the uremic syndrome. This syndrome leads to death unless the toxins are removed by renal replacement therapy, using dialysis or kidney transplantation **(Jameson, 2010).** In CRF, there is a persistent and irreversible reduction in the overall renal function. Not only the excretory functions are disturbed but also the endocrine and the haemopoietic functions as well as the regulation of acid-base balance become abnormal., These derangements in the internal environment (internal milieu) of the body will result in the uremic syndrome **(Sobh, 2000).** In CRF, the metabolic consequences of uremia are slowly progressive in nature **(Glassock, 1987).**

Chronic acidosis can affect myocardial contractility; contribute to central nervous system toxicity. Water and salt intake persistently exceeds excretory capacity, edema formation occurs. Chronic sodium retention can manifest as persistent arterial hypertension. Kidney also fails to convert 25-hydroxyvitamin D to the metabolically active 1, 25-dihydroxyvitamin D, resulting in the defective absorption of calcium from the intestinal tract; secondary hypocalcemia induces secondary hyperparathyroidism with concomitant demineralization and reabsorption of bone. Depression of red cell production is the consequence of decreased renal production of the hormone erythropoietin. Increased red blood cell destruction resulting from uremic toxins as well as the mechanical damage to red cell observed in a variety of glomerular disease. Finally, patients with CRF had depressed cellular immunity and humoral immunity **(Schreiner & Kissane, 1990).**

2.2 Chronic kidney disease

2.2.1 Definition of chronic kidney disease

CKD is a worldwide public health problem, with adverse outcomes of kidney failure, cardiovascular disease (CVD), and premature death **(Levey et al., 2005).** CKD encompasses a spectrum of different pathophysiologic processes associated with abnormal kidney function, and a progressive decline in GFR, based on recent guidelines of the national kidney foundation [kidney dialysis outcomes quality initiative (KDOQI)], in which stages of CKD are defined according to the estimated GFR **(Jameson, 2010).**

The KDOQI definition and classification were accepted, with clarifications. CKD is defined as kidney damage or GFR < 60 mL/min/1.73 m^2 for three months or more, irrespective of cause. kidney damage in many kidney diseases can be ascertained by the presence of albuminuria, defined as albumin-to-creatinine ratio > 30 mg/g in two of three spot urine specimens. GFR can be estimated from calibrated serum creatinine and estimating equations, such as the modification of diet in renal disease (MDRD) study equation or the Cockcroft-Gault formula. Kidney disease severity is classified into five stages according to the level of GFR. Kidney disease treatment by dialysis and transplantation should be noted. Simple, uniform classifications of CKD by cause and by risks for kidney disease progression and CVD should be developed (**Levey et al., 2005).**

2.2.2 Glomerular filtration rate

GFR is one of the most important parameters for assessment of renal function and considered the best marker of renal function (**Villa et al., 2005.; and K/DOQI, 2016**). It's measured as the renal clearance of a particular substance from plasma and is expressed as the volume of plasma that is completely cleared of that substance in 1 min (**Renal Date System, 2001; K/DOQ1, 2002 and Gross et al., 2005**). GFR is a direct measurement of kidney function and is reduced before the onset of symptoms of kidney failure. The ideal filtration marker for GFR would appear endogenously in plasma at a constant rate, be freely filtered across the capillary wall, be neither secreted nor reabsorbed by the renal tubule, and undergo no extra-renal elimination (**Renal Date System, 2001; K/DOQ1, 2002 and Gross et al., 2005**). GFR is an important indicator of kidney function, critical for detection, evaluation and management of CKD (**Levey et al., 2006.; Stevens et al., 2006 and Stevens. et al., 2008).** It's the best overall index of kidney function. Decreased GFR is associated with increased risk of complications related to kidney disease, including uremic manifestations of kidney disease, acute kidney injury(AKI), kidney failure, and CVD (**Stevens et al., 2011**)

A low or decreasing GFR is a good index of CKD. Since the total kidney GFR is equal to the sum of the filtration rates in each of the functioning nephrons, the total

GFR can be used as an index of functioning renal mass (**White et al., 2010 and Delanaye et al., 2010**). (**Table: 2.2).** illustrate normal GFR.

Table (2.2): Normal glomerular filtration rate

Age	Mean GFR±SD (mL/min/1.73 m^2)	
1 week (males and females)	41±15	
2–8 weeks (males and females)	66±25	
8 weeks – 2 years (males and females)	96±22	
2–12 years (males and females)	133±27	
	Male	**Female**
13–20 years	140±30	126±22
21–29 years	128±26	118±24
30–39 years	110±23	107±21
40–49 years	100±21	97±19
50–59 years	90±19	86±17
60–69 years	80±16	75±15
70–79 years	70±14	64±13
>80 years	50±12	43±11

Adopted from (NKF: K/DOQI, 2002**).*

GFR cannot be practically measured for routine clinical or research purposes and therefore, serum creatinine is often used to estimate GFR. Several factors affect the level of serum creatinine other than GFR, including its generation from muscle metabolism (**Levey et al., 2006.; Stevens et al., 2006 and Stevens. et al., 2008).**

Serum creatinine is the most commonly used biochemical parameter to estimate GFR in routine practice. However, there are some shortcomings to the use of this parameter. Factors such as muscle mass and protein intake can influence serum creatinine, leading to an inaccurate estimation of GFR. Normal serum creatinine may be observed in individuals with significantly impaired GFR (**Levey et al., 1988 and Villa et al., 2005**). Moreover, in unstable, critically ill patients, acute changes in renal function can make real-time evaluation of GFR using serum creatinine difficult (**Levey et al., 1988 and Villa et al., 2005**). GFR can be measured by clearance techniques involving endogenous (e.g., creatinine and urea) or exogenous (e.g., inulin, iohexol, and iothalamate) filtration markers, with the latter

considered to be the gold-standard approach **(K/DOQI, 2016).** Unfortunately, clearance measurements are both cumbersome and costly; thus, in clinical practice, GFR is often estimated based upon the serum creatinine concentration **(Levey et al., 2000).** The measurement of creatinine clearance eliminates some of the problems with serum creatinine level and also improves the assessment of GFR, but it presents some disadvantages, as listed in **(Table: 2.3)**. Other measurements using the infusion of external substances, such as inulin, radionuclides, or iohexol, have been proposed for determining GFR, but they are difficult to perform, costly, in some cases require radiation exposure, have difficult and time-consuming methods for analysis, and are impractical for routine GFR assessment. Therefore, it would be of great value to find a serum marker able to detect renal function impairment, especially at the initial phase **(Coll et al., 2000)**. Serum creatinine and urea levels and creatinine clearance are currently used in the assessment of GFR. These markers are rapidly and easily performed in the clinical laboratory. Moreover, the precision and reliability of each measurement method are known and well documented **(Coll et al., 2000)**.

Table (2.3) markers of glomerular filtration rate and production and elimination factors that could affect its determination and interferences

Marker	Production	Elimination	Dependent on	Interferences
BUN	Liver	GFR, Skin Passive tubular reabsorption Gastrointestinal tract	Protein intake Nitrogen metabolism Plasma renal flow	**Positive:** aminosalicyclic acid, bilirubin, hemoglobin, uric acid, sulfonamides, tetracycline. **Negative:** ascorbic acid, levodopa, streptomycin
Creatinine	Muscle	GFR Tubular secretion	Muscle mass Meat intake	**Positive:** glucose, fructose, pyruvate, Uric acid, protein, bilirubin, cephalosporin
Creatinine Clearance			Tubular secretion Error collecting urine samples, incorrect storage of urine samples	

*BUN, Blood urea nitrogen.   **Adopted from (Coll et al., 2000).**

The GFR is traditionally considered the best overall index of renal function in health and disease. Because GFR is difficult to measure in clinical practice, most clinicians estimate the GFR from the serum creatinine concentration. However, the accuracy of this estimate is limited because the serum creatinine concentration is

affected by factors other than creatinine filtration (**Osman and Elmadani., 2014**). To circumvent these limitations, several formulas have been developed to estimate creatinine clearance from serum creatinine concentration, age, sex, and body size (**Levey et al., 2006 and Stevens et al., 2006).** Numerous GFR estimating equations have been developed, the most widely used of which was derived from the MDRD Study (**Levey et al., 2000),** GFR is widely estimated by serum creatinine based equations of Cockcroft-Gault standardized for body surface, and MDRD formula. Both equations take parameters of serum creatinine, age, and gender into account. As creatinine production is affected by age, muscle mass, gender, medications, and catabolic state, the serum cys-C based equations were proposed for GFR estimation (**Van Deventer et al., 2011., and Donadio et al., 2012**), especially because it has been recently shown that ethnicity coefficients did not seem to be necessary (**Teo et al., 2012**).

No formula is more widely used to predict creatinine clearance than that proposed by Cockcroft and Gault (**Cockcroft and Gault, 1976),** this formula is used to detect the onset of renal insufficiency, to adjust the dose of drugs excreted by the kidney, and to evaluate the effectiveness of therapy for progressive renal disease. More recently, it has been used to document eligibility for reimbursement from the Medicare ESRD Program (**Levey et al., 1999**), the MDRD Study, a multicenter, controlled trial, evaluated the effect of dietary protein restriction and strict blood pressure control on the progression of renal disease (**Levey et al., 1999**).

GFR estimating equations, such as the MDRD Study equation, include age, sex, and race to account for average differences in muscle mass in subgroups; however, the magnitude of the association of muscle mass with age, sex, and race varies among populations, compromising the generalizability of the equations. Furthermore, incorporation of age, sex, and race in the estimating equation does not account for variation in creatinine generation caused by diet or other clinical conditions, such as illnesses complicated by malnutrition, inflammation, or deconditioning, that also affect muscle mass. These other causes of creatinine generation lead to imprecision in the estimates (**Levey et al., 2006 and Stevens et al., 2006).** GFR is measured in absolute values ($mL*min^{-1}$) or in relative values after corrected for body surface area [$mL*min^{-1}*(1.73m^2)^{-1}$](**Thomas and Huber, 2006).**

Essentially all GFR estimating equations have been developed from cross-sectional data and perform well when used to classify individuals at single points in time, particularly for levels of GFR less than 60 ml/min per 1.73 m^2. Ideally, these equations could also be used to monitor GFR changes over time in research and clinical practice **(Xie et al., 2008)**, a decline in GFR correlates with the pathologic severity of renal disease.If GFR decreases below 15 [ml$_*$min^{-1}$_*$(1.73 m^2)$^{-1}$], replacement therapy with dialysis is then necessary. However, the level of GFR can be insensitive to detection of a loss of nephron numbers **(Manjunath et al., 2001 and Thomas & Huber., 2006).**

A decrease in GFR precedes kidney failure in all forms of progressive kidney diseases. Monitoring changes in GFR can delineate the progression of kidney disease. The level of GFR is a strong predictor of the time of onset of kidney failure as well as the risk of complications of CKD **(National Kidney Foundation (NKF), 2002 & Mungrue et al., 2016).** The level of GFR should be estimated from prediction equations that take into account the serum creatinine concentration and some or all of the following variables: age, gender, race, and body size **(Table: 2.4).** The following equations provide useful estimates of GFR: In children, the Schwartz and Counahan-Barratt equations. In adults, the abbreviated MDRD Study equation and Cockcroft-Gault equations **(NKF: K/DOQI, 2002).**

Table (2.4): Prediction of glomerular filtration rate based on serum creatinine.

Equation Author	Equation
Schwartz	GFR (ml/mim/1.73m²) = 0.55×length ∕ Scr
Counahan-Barratt	GFR (ml/mim/1.73m²) = 0.43 × length ∕ Scr
Abbreviated MDRD Study	GFR(ml/mim/1.73m²)=186×(Scr)×(Age)×(0.742 if female) × (1.210 if black)
Cockcroft-Gault	Ccr (ml/min) = (140 – Age)×Weight ×(0.85 if female)∕72× Scr

Scr: serum creatinine, **Ccr:** creatinine clearance. **Adopted from (NKF: K/DOQI, 2002).**

2.2.3 Proteinuria

Proteinuria is a marker of kidney damage and an important risk factor for progression of CKD as well as CVD morbidity and mortality **(Ahmed et al., 2013)**. Proteinuria is often transient and benign, but persistent proteinuria is not only a

marker of early kidney disease, but also an independent risk factor for atherosclerotic diseases, such as coronary or cerebrovascular arterial diseases **(Perkovic et al., 2008).** Proteinuria is associated with more rapid progression of CKD and a greater likelihood of developing ESRD. Consequently, detection and quantitation of proteinuria are essential to the diagnosis and treatment of CKD **(Jafar et al., 2003)**. Screening for proteinuria often alerts the physician to the presence of CKD before changes in the GFR become apparent. Significant kidney disease can present with decreased GFR or proteinuria, or both **(Garg et al., 2002)**. GFR below 30 mL per minute per 1.73 m^2, had no proteinuria. Therefore, an estimate of the GFR and a screening method for proteinuria are required **(NKF: K/DOQI, 2002)**. The incidence of proteinuria in randomly collected urine specimens increases with age and is significantly associated with increased mortality **(Sureshkumar et al., 2003).**

Albumin is the principal component of proteinuria in glomerular disease. The presence of persistent albumin in the urine is a clear sign of glomerular abnormality. Microalbuminuria describes the urinary excretion of small amounts of albumin which identifies the very early stage of diabetic kidney disease. The albumin creatinine ratio is the preferred method of detecting microalbuminuria. There is strong evidence that treatment in the early stages of CKD reduces progression of kidney damage **(Ahmed et al., 2013)**, normally urine contains less than 150 mg protein per day, with only 20% of it as albumin (less than 30 mg/d or 20 μg/min) and 40% as Tamm-Horsfall mucoproteins, which are secreted by the distal tube **(Diamantis et al., 2008).**

Proteinuria is defined by the presence of excessive amounts of protein in the urine (approximately >150mg/24 hours). Proteinuria with more than 3500 mg/24 hours is called nephrotic range proteinuria, which usually represents glomerular disease **(Kashif et al., 2003 and Berggard, 2004).**

Microalbuminuria is defined as a urinary excretion of albumin that is above normal (20ug/min or 30mg/24 hours) but is below the sensitivity of conventional test strips (300mg/24 hours). Microalbuminuria is recognized to be an early marker for nephropathy associated with type 2 diabetes mellitus or hypertension, and also is an

independent marker for CVD. Albuminuria of more than 300mg/24 hour is called macroalbuminuria **(Lindeman et al., 1998 Berggard, 2004).**

Albuminuria is defined as an ACR of 30 mg/day or higher, with microalbuminuria defined as an ACR of 30 to 300 mg/day, and macroalbuminuria defined as an ACR over 300 mg/d **(Johnson, 2012 & Berggard, 2004). (Table: 2.5)**

Table (2.5): Proposed definitions of proteinuria and albuminuria

	Microalbuminuria	**Albuminuria (Macroalbuminuria)**	**Proteinuria**
Per 24 hours	30–300 mg/d	>300 mg/d	>150–300 mg/d
Dipstick	>3 mg/dL (Albumin specific dipstick)	>20 mg/dL	>30 mg/dL
Random urine ACR or PCR g/mmol	**M:** >1.9 g/mmol, > 17 mg/g **F:** >2.8 g/mmol, > 25 mg/g	**M:** >28 g/mmol, > 250 mg/g **F:** >40 g/mmol , > 355 mg/g	**M:** >28g/mmol, > 250 mg/g **F:** >40 g/mmol, > 355 mg/g

PCR: Protein/Creatinine ratio.   **ACR:** Albumin/Creatinine ratio

Adopted by (**Warram et al., 1996 and CARI Guidelines, 2016)**

Table (2.6): stages of CKD should be based on kidney damage (albuminuria/proteinuria), irrespective of the underlying diagnosis

Kidney damage stage	**Urine albumin/ creatinine ratio (mg/mmol)**	**24h urine albumin (mg/day)**	**Urine protein: creatinine ratio (mg/mmol)**	**24h urine protein (mg/day)**
Normoalbuminuria	**M**: < 2.5 **F**: < 3.5	<30	**M**: < 4 **F**: < 6	<50
Microalbuminuria	**M**: 2.5-25 **F**: 3.5-35	30-300	**M**: 4-40 **F**: 6-60	50-500
Macroalbuminuria	**M**: > 25 **F**: > 35	>300	**M**: > 40 **F**: > 60	>500

Adopted from (Johnson, 2012).

Proteinuria is one of the most frequent modes of presentation of underlying renal disease, and it is not only an early marker of kidney damage, but also a guide to differential diagnosis, prognosis, and treatment **(National Kidney Foundation KDOQI, 2002).** In particular, detection of an increase in protein excretion is known to have both diagnostic and prognostic value in the initial detection and confirmation of renal disease, and the quantification of proteinuria can be of considerable value in assessing the effectiveness of therapy and the progression of the disease **(Price et al., 2005 and CARI Guidelines, 2016).** nephrotic range proteinuria is associated with a wide range of complications, including hypoalbuminemia, edema, hyperlipidemia,

and hypercoagulability; faster progression of kidney disease; and premature CVD. However, it is now known that sub-nephrotic range proteinuria is also associated with faster progression of kidney disease and development of CVD (**National Kidney Foundation KDOQI, 2002).**

2.2.4 Classification of chronic kidney disease

CKD has been classified into various stages for the purpose of prevention, early identification of renal damage and institution of preventive measures for progression of the primary damage and appropriate guidelines for instituting management for prevention of complications in severe CKD (**Vijayakumar et al., 2007).**

National kidney foundation (NKF) classified CKD into five stages according to the level of GFR (**Table: 2.7).** For stages 1 and 2, kidney damage was assessed by spot albumin-to-creatinine ratio, the stage is defined by the level of GFR, with higher stages representing lower GFR levels (**National Kidney Foundation KDOQI, 2002).**

Table (2.7): Classification of the stages of chronic kidney disease and abnormalities

Stages of kidney disease	GFR (mL/min/1.73 m^2)	Description	Related abnormalities
Normal	**> 90**	Healthy kidney	
Stage 1	**$\geq$ 90**	Normal or increased GFR	Albuminuria, proteinuria, hematuria
Stage 2	**60-89**	Normal or slightly decreased GFR	Albuminuria, proteinuria, hematuria
Stage 3A	**45-59**	Mild-moderate decrease in GFR	Proteinuria, hematuria, anemia, hypocalcemia, hyperphosphatemia
Stage 3B	**30-44**	Moderate-severe decrease in GFR	
Stage 4	**15-29**	Severe decrease in GFR	Proteinuria, hematuria, anemia, acidosis, hypocalcemia, hyperphosphatemia
Stage 5	**<15 or on dialysis**	Very severe or End-stage kidney failure	Uremia, anaemia, malnutrition, hyperparathyroidism, high B.P., swelling in hands/legs eyes/lower back, shortness of breath

Adopted from (NKF: KDOQI, 2002 and Johnson, 2012).

Measurements of urinary sediments, markers of renal damage, renal imaging and renal pathologic abnormalities can help identify kidney diseases where the GFR is not significantly altered. Therefore, the CKD Stages 1 and 2 are defined as kidney damage, which are measured by using urinary, imaging or pathologic methods, with normal or increased GFR ($\geq$ 90 mL/min/1.73m^2) and with mildly decreased GFR

(60-89 mL/min/1.73m^2). The CKD Stages 3-5 are defined mainly by GFR measurements (30-59 mL/min/1.73m^2, 15-29 mL/min/1.73m^2, and <15 mL/min/1.73m^2), respectively the CKD staging system has some issues and it has received some major criticisms **(Polkinghorne, 2011).** It does not consider the underlying pathophysiology of renal failure. Furthermore, proteinuria (protein in the urine) is an important renal prognostic indicator the current CKD staging system does not take it into account. Therefore, have proposed a new classification system based on both the GFR and the proteinuria level **(Tonelli et al., 2011)**, this classification has not yet been widely adapted. For physicians and health care providers, it is important to know that the management of CKD patients should be individualized and should not be based solely on the CKD stages **(Tonelli et al., 2011)**. The podocytes probably form the most important filtration barrier for plasma proteins **(Scott and Quaggin, 2015).**

In normal conditions, urine formation starts when the blood from the afferent arterioles filtrates through the glomerular capillaries that consist of a filtrate with small plasma proteins and electrolytes. The amount of the filtered blood per time interval normalized to an idealized body surface area (1.73m^2) is called the GFR (GFR, mL/min/1.73m^2), which is determined by the hydrostatic pressure and colloid osmotic (oncotic) pressure across the capillary membrane, and the capillary filtration coefficient in the glomerulus **(Guyton and Hall, 2000).** It is important to identify factors that are associated with an increased risk of developing CKD, so that screening programmed can be targeted at high-risk groups. Risk factors relating to CKD can be divided into two main groups: initiating factors that increase the risk of developing CKD; and perpetuating factors that increase the risk of CKD progression to ESRD **(Taal and Brenner, 2006). (Table: 2.8)**

Table (2.8): CKD initiating and perpetuating factors

Initiating factors	Perpetuating factors
Increasing age	Africane American race
Gender	Proteinuria
Ethnicity	Hypertension
Genetics -Family history of CKD	High dietary protein intake
Socio-economic status	Obesity
Metabolic syndrome	Anaemia
High normal urinary albumin excretion	Dyslipidaemia
Dyslipidaemia	Nephrotoxins
Nephrotoxins (NSAIDs, antibiotics, radiological contrast, light chains)	Smoking
Primary renal disease	CVD
Urological disorders (obstruction, recurrent urinary infections)	
CVD	
Diabetes mellitus	
Abnormal Mineral and Bone Metabolism	
Malnutrition	
CKD, chronic kidney disease; **NSAIDs**, non-steroidal anti-inflammatory drugs.	

Adopted by (Taal & Brenner, 2006 and Evans & Taal, 2011).

2.2.5 Genetic factors

Hereditary renal diseases that result from single gene defects (e.g. polycystic kidney disease, Alport's disease and Fabry's disease) make up only a small proportion of cases. More significant are genetic factors that increase the risk of developing multi-factorial CKD in a person with a family history of CKD (**Lei et al., 1998**).

The GFR measurement is the overall GFR in one or both kidneys in a subject. A normal GFR in an adult is approximately 125 mL/min (**Koeppen and Stanton, 2012**). Therefore, an average adult has a GFR of 180 L/day. If this filtered fluid is not reabsorbed, the patient will lose a significant number of electrolytes. As a result, the majority of the filtered electrolytes (such as sodium and potassium) and plasma fluid are reabsorbed. Currently, kidney function is reported based on GFR, either measured or estimated. The ideal markers for assessing GFR should be freely filtered by glomeruli without tubular secretion and reabsorption (**Guyton and Hall, 2000).**

The renal filtration fraction (FF) is another variable that is closely linked to GFR. FF is the amount of renal plasma flow that is filtered. It is calculated by the following equation below (**Koeppen and Stanton, 2012**).

$$\text{Filtration Fraction (FF)} = \frac{\text{GFR}}{\text{Renal Plasma Flow}}$$

A normal FF is approximately $18.7 \pm 3.2\%$ in healthy young adults between the ages of 20-30 years (**Huseman et al., 1999**), when the FF is above the reference interval of 18- 22%, it is considered hyperfiltration (**NIH-Clinical Center, 2011**). Hyperfiltration can occur at the individual glomerular level in a situation where GFR is decreased. Early development of diabetic nephropathy is commonly associated with hyperfiltration and is a maladaption process, this can lead to poor renal outcome . reducing hyperfiltration through medications, such as angiotensin receptor blockers or angiotensin converting enzyme inhibitors, may prevent or reduce the rate of renal function decline (**Wiseman et al., 1987 and Mogensen, 2008**).

2.2.6 Epidemiology and etiology of chronic kidney disease

CKD has emerged as a global public health burden for its increasing number of patients, high risk of progression to ESRD, and poor prognosis of morbidity and mortality (**El Nahas and Bello , 2005 and Levey et al., 2007**). It attracts worldwide attention to its epidemiology, risk factors, treatment plans and preventive actions (**Levey et al., 2009**).

Estimated GFR has become a standard method to evaluate CKD based on diagnostic criteria and classification by the NKF, USA (**NKF: K/DOQI, 2002**). CKD is a common condition, associated with a significantly increased risk of hospital admission, morbidity and death due to CVD. CKD can also progress to ESRD, which results in patients requiring dialysis and/or renal transplantation, together termed renal replacement therapy (RRT) (**Evans and Taal., 2011**). CKD estimated to affect 10%-15% of adult populations for which data are available, has come to be recognized as an international public health concern (**Chadban et al., 2003.; Hallan et al., 2006.; Coresh et al., 2007 and Perkovic et al., 2008**).

CKD has complicated interrelationship with diabetes and hypertension and other associated diseases, and it is an independent risk factor for CVD and all-cause mortality **(Snively & Gutierrez, 2004 and Weiner et al., 2004).** The Outcomes of CKD include not only progression to ESRD but also complications of reduced kidney function, such as hypertension, malnutrition, anemia, bone disease and a decreased quality of life. The enormous costs of treatment of the associated morbidity including ESRD lead to a large burden for the health care system worldwide **(Collins et al., 2005).** After the kidney disease outcome quality initiation (K/DOQI) clinical practice guideline for definition and classification of CKD have been published, more epidemiologic data about prevalence of CKD in the general population are available. However, few studies focused on risk factors for early stages of CKD among older adults. **(Levey et al., 2005).** the risk of progression to ESRD requiring dialysis or kidney transplant, CKD is now known to be associated from its earliest stages with significantly increased risks of CVD morbidity, premature mortality, and decreased quality of life **(Chow et al., 2003.; Go et al., 2004 and Keith et al., 2004).** Accordingly, early detection by screening of high-risk individuals in primary care, monitoring changes in kidney function over time, and management of comorbid cardiovascular risk factors are increasingly advocated **(NKF: K/DOQI, 2002).**

The ESRD is increasing worldwide. RRT and kidney transplantation are increasing the burden on health systems **(Ghonemy et al., 2016)**. This condition is particularly serious in developing countries where health resources are inadequate **(Stengel et al., 2003)** Worldwide, the number of patients receiving RRT is estimated at more than 1.4 million, with the annual incident rate growing to 8% **(Schieppati et al., 2005).** ESRD has many causes that vary from one patient to another. The key risk factors for CKD are the increasing age of the population, diabetes mellitus and hypertension and medications, such as the use of analgesics regularly over long durations of time resulting in analgesic nephropathy and kidney damage. Polycystic kidney disease is an example of a hereditary cause of CKD**.** Diabetes is the largest single cause of ESRD in the United Kingdom, accounting for 30-40 % of all cases **(Sandra et al., 2005).**

In many Arab countries, obstructive uropathy constitutes a major cause of ESRD (40%). The two most common underlying causes are renal calculi and schistosomiasis. In many developing countries, chronic glomerulonephritis is often caused by infections and infestations, and is a leading cause of CKD (**Ulasi et al., 2006**). The body of evidence for other modifiable risk factors such as lifestyle factors is growing as some studies suggest that tobacco use is positively associated with CKD (**Shankar et al., 2006**). Alcohol has been linked as a cause of kidney disorders in some clinical and experimental studies (**Schaeffner et al., 2005**). Also, obesity seems to be an important-and potentially preventable-risk factor for CRF. (**Ejerblad et al., 2006**).

Worldwide, the prevalence of ESRD differs greatly. According the united states renal data system, the highest prevalence was found in Taiwan, with 2447 patients per million population (pmp), and the lowest prevalence was in Philippines, at 110 pmp. In the United States, the prevalence was 1811 pmp (**United States Renal Data System, USRDS, 2011**). In Europe, the prevalence has increased from 760 pmp in 2004 to 889 pmp in 2008 (**Stel et al., 2011**). In Palestine, **Khader et al., 2013**), were reported the prevalence of patients with ESRD on dialysis during the study period was 240.3 pmp and they showed the highest prevalence was seen in Jericho city. There were 57.7% males and 42.4% females in the study. The majority of patients (62.3%) were living in villages, while 28.8% were living in cities and 8.9% were living in refugee camps. Most of the patients (45%) were aged between 45 and 64 years. The vast majority of patients were either diabetic (22.5%) or hypertensive (11.1%) or both at the same time (10.6%). There were a considerable number of patients in whom the cause was undetermined (27.6%). The majority of recorded cases of congenital causes were from the Hebron, Jenin and Tubas districts. The prevalence of ESRD noted in the study was comparable with other regional countries but far below the rate recorded in industrialized countries. In the Palestinian territories, there is a general lack of national statistics and surveys, particularly in the public health section. Increased efforts and awareness should be focused on the prevention and treatment of diabetes mellitus and hypertension as they are the main causes of ESRD. There should also be an additional enhancement and implementation of strategies for the registration of data in order to conduct

periodic comparisons and analytical studies to improve the management and quality of life of ESRD patients. Most common causes of chronic renal failure in Jenin district were diabetes mellitus (33.3%), hypertension (16.7%), and chronic glomerulonephritis (13.1%). Inherited kidney diseases formed an important percentage (17.9%) and included primary hyperoxaluria (10.7%), Alport's syndrome (5.9%), and adult polycystic kidney disease (1.2%) **(Abumwais et al., 2012).**

In children there is a wide range of conditions and causes of CKD such as: Intrauterine infections, drugs intake in early pregnancy, genetic kidney disease, and congenital anomalies of kidney, postnatal infections, metabolic diseases, and nephrotoxic drugs **(Vijayakumar et al., 2007 and Fathallah-Shaykh et al., 2015).**

There are no recent data about the prevalence of ESRD; The prevalence of both acute and CRF is high in the Arab world. Data available on the exact prevalence of various renal diseases are very limited. Nevertheless, the reported prevalence of CRF is 80 to 120 per million population (pmp) in the Kingdom of Saudi Arabia and 225 pmp in Egypt **(Shaheen and Khader, 2005).** In Saudi Arabia **(Mohamed et al., 2004**) indicated an increase in the incidence of ESRD from 6.52 per 100000 populations in the 1988 to 13.75 per 100000 populations in the 2001. however, the last statistics was performed by Palestinian Health Information Center in 2005, with prevalence of renal failure was 4% with an incidence of 10.8 per 100,000, distributed as 1.1% in Gaza strip and 2.9% in the West Bank **(Palestinian Health Information Center, PHIC, 2005.)**

2.2.7 Causes of end stage renal disease

ESRD has many causes that vary from one patient to another. The most common causes include old age, uncontrolled hypertension, CVD, glomerulonephritis, atherosclerosis, obstruction of the urinary tract by stones or cancer, diabetes mellitus, obesity, family history of stage 5 CKD or hereditary kidney disease eg, polycystic kidneys, medications such as the use of some analgesics regularly over long durations of time, infective, obstructive and reflux nephropathies, hypercalcemia, neoplasms, myeloma and multisystem diseases with potential kidney involvement eg, systemic lupus erythematosus (SLE) **(Sandra, 2005; Hyman, 2006;**

Soyibo and Barton, 2007; Hartmann et al., 2009; Herzog et al., 2011; Levey and Coresh, 2012 and NICE Clinical Guidelines, 2014).

2.2.8 Treatment of end stage renal disease

The most important treatment alternatives for ESRD include hemodialysis (HD), peritoneal dialysis (PD) and kidney transplantation. The populations of ESRD patients, dialysis patients and patients living with a transplanted kidney have increased steadily over the past years, whereby consistently more than three quarters of all ESRD patients were treated by dialysis **(Fresenius Medical Care, 2011).**

2.3 Methods of measuring glomerular filtration rate

Over the last 80 years, several methods have been developed to assess GFR. Each of these methods has its pros and cons - some are more invasive and time consuming, while others may not be sufficiently sensitive or specific.

2.3.1 Exogenous biomarkers to estimate glomerular filtration rate

2.3.1.1 The inulin study

Inulin is a polysaccharide, widely distributed as a reserve material in many plants, which on hydrolysis yields a fructose polymer made from the Jerusalem artichoke that does not have non-renal elimination, no plasma protein binding, and is neither absorbed or excreted by the tubule. It has the characteristics of an ideal renal marker for GFR measurements **(Richards et al., 1934.; and Shannon & Smith, 1935).** Therefore, inulin clearance is considered the gold standard for measuring GFR. This method was initially developed in 1935 by Homer Smith **(Shannon & Smith, 1935 and Filler & Sharma, 2008).**

In the traditional method, an intravenous infusion of inulin is given after a bolus injection until a steady state is reached. Urinary inulin clearance is then measured. Because it is time consuming, modified versions of the traditional inulin clearance were developed. Several hours of inulin infusion are the ideal method for measuring clearance, and to minimize error, catheterization is best for accurate urine collection. Despite the use of these methods, there is a 10% inter-assay variability with inulin measurements due to analytical challenges and the inhomogeneity of the biomarker, especially when older biochemical methods are employed, rather than

mass spectrometry (**Filler and Sharma, 2008).** Because of its invasiveness (catheterization) and difficulties with the availability of inulin, an inulin study is rarely performed and is limited to a research setting **(Cherney et al., 2010).**

2.3.1.2 The nuclear glomerular filtration rate study

In the 1970's, nuclear medicine techniques replaced the inulin clearance method. The techniques, which use radio-labeled markers that have similar properties to inulin, have produced findings comparable to inulin GFR clearance studies of patients with GFRs above 20 mL/min/1.73m^2 **(Rehling et al., 1984 and Morton et al., 1997).** A bolus of clearly measured and suitable compound, which was injected through a venipuncture, is commonly utilized. The rate of decreasing plasma concentration of the compound, after adjusting for its inherited decay rate, is measured and is used to calculate renal GFR. In Europe, chromium 51-labeled ethylenediaminetetraacetate (^{51}Cr-EDTA) is the most widely used radio-labeled isotope. Technetium ^{99m}-labeled diethylenetriaminepentaacetic acid (^{99m}Tc-DTPA) is the most commonly used GFR marker in North America **(Chantler and Barratt, 1972.; Picciotto et al., 1992 and Filler et al., 2002).** Although some studies have observed systematic differences between (^{51}Cr-EDTA) and (^{99m}Tc-DTPA), these differences are small and (^{99m}Tc-DTPA) is recommended as an acceptable alternative to (^{51}Cr- EDTA) **(Rehling et al., 1984.; Fleming et al., 1991.; Biggi et al., 1995.; Filler et al., 2002.; Fleming et al., 2004 and Fotopoulos et al., 2006).**

Other exogenous markers that have been utilized are [125]Iodine iothalamate, iothalamate and iohexol. The latter two have been used without being radiolabeled. All markers except iothalamate have a small amount of plasma protein binding **(Filler and Sharma, 2008).** There are certain compartmental methods where the timing of the blood sampling can result in different values. It has been considered that the slope-intercept method, restricting the blood samples to the second of the two exponential components, provides the best compromise between accuracy and reliability, in addition to its simplicity **(Chantler et al., 1969).**

2.3.2 Endogenous biomarkers to estimate glomerular filtration rate

Although using exogenous markers, such as inulin and nuclear isotopes, to measure GFR is considered more accurate; the method is invasive and time

consuming, and not practical for day-to-day use (**Berg et al., 2015**). Therefore, endogenous biomarkers are commonly used to estimate. The ideal biomarkers in patients with CKD should confirm the level of renal function, measure the total "renal clearance" and predict the outcomes of "renal health". Once the potential biomarkers are identified, they need to go through vigorous development and testing. In stage 1, pre-clinical research identifies promising markers that require further exploration. In stage 2, the potential biomarker is tested in human beings to determine if it can distinguish individuals severely affected with the disease from those who are healthy. In stage 3, retrospective studies establish whether the biomarker detects disease before the clinical diagnosis becomes evident. In stage 4, the biomarker undergoes prospective evaluation to determine the performance characteristics of the test in a setting in which it will be clinically applied. Finally, in stage 5, the focus is on the use of biomarkers to assess in the natural course of illness (**Baek et al., 2012 and Rysz et al., 2012**). When biomarkers are used for screening, it should be shown in randomized controlled trials that the application of interventions earlier in the process is indeed beneficial (**Vafeiadou et al., 2015**). Several biomarkers have been used to estimate glomerular filtration rate and have gone through at least stage 3 or 4; they include small plasma solutes such as creatinine, and endogenous small molecular weight proteins such as cys-C, beta-trace protein and B_2 microglobulin (**Juraschek et al., 2013 and Ebert et al., 2017).**

2.3.3 Methods of estimating glomerular filtration rate biomarkers

2.3.3.1 Creatinine

Creatinine (Cr) is a small molecular weight solute (113 Dalton) (**Okuda et al., 2008**)**.** it is the product of creatine and phosphocreatine, and is filtered by glomeruli. and could be used for assessing kidney function, there are various methods and reference ranges for serum creatinine measurements (**Horio and Orita, 1996**)**.** Recently, the isotope dilution-mass spectrometry (IDMS) reference method has improved and standardized the accuracy of creatinine measurements by eliminating some of the analytical problems (**Cobbaert et al., 2009**)**.** Measuring the creatinine clearance (mL/min/1.73m^2) using 24-hour urinary creatinine measurements approximate GFR. However, it is not a true measure of GFR because there is some

tubular secretion of creatinine. The equation for calculating 24-hour urinary creatinine clearance (CrCl) is as follows: **(Koeppen and Stanton, 2012).**

$$CrCl = \frac{Ucr \; x \; \dfrac{1000 \; x \; Vurine}{1440}}{Pcr} \; x \; \frac{1.73}{BSA}$$

where (UCr) (mmol/L) is the urinary creatinine concentration, (V urine) is the urinary volume (L/24 hours), (PCr) (mmol/L) is the plasma creatinine concentration and (BSA) is the body surface area. This method is not routinely used because collecting 24-hour urinary creatinine clearance is cumbersome for patients. Timed urine collections are also notoriously inaccurate. Furthermore, creatinine is secreted by tubule. Therefore, creatinine clearance overestimates GFR by approximately 10% of the total excretion **(Koeppen and Stanton, 2012).**

A method called cimetidine creatinine clearance, where cimetidine treatment is used to block tubular secretion of creatinine, is impractical., To adjust for the problems, an equation to estimate (CrCl) was developed by Cockcroft and Gault (CG-CrCl) and was published in 1976 **(Cockcroft and Gault, 1976).**

$$CG \; CrCl = \frac{(140 - Age) \; x \; (weight \; in \; Kg) \; x \; (0.85 \; if \; Female)}{72 \; x \; Serum \; creatinine}$$

The Cockcroft-Gault equation requires the weight of the patients, whereas some of the estimated GFR equations do not. Therefore, it is much easier to generate laboratory GFR results using the MDRD study equation along with the creatinine values. There have been more published studies on using other creatinine-based estimating GFR (eGFR) equations than the Cockcroft-Gault equation. As a result, the Cockcroft-Gault equation is not as commonly used. There are several different eGFR equations. In the pediatric population, the most commonly used equation is the Schwartz equation **(Schwartz et al., 1976).** In the adult population, there are a few commonly used eGFR equations. See **(Table: 2.9):** for a summary.

Table (2.9): Commonly used creatinine-based estimating glomerular filtration rate equations

Equations (GFR mL/min/1.73m^2)	
Original MDRD[39]	eGFR = $170(Scr)^{-0.999}Age^{-0.176}BUN^{-0.170}Alb^{0.318}(0.762 \, if \, F)(1.180 \, if \, A.A.)$
Abbreviated MDRD[39]	eGFR = $175(Scr)^{-1.154}Age^{-0.203}(0.742 \, if \, F)(1.212 \, if \, A.A)$[*]
CKD-EPI[48]	eGFR = $141 \left(min\left(\frac{Scr}{K,1}\right)^a max\left(\frac{Scr}{K,1}\right)^{-1.209}\right)0.993^{Age}(1.018 \, if \, F)(1.159 \, if \, A.A.)$[**]
Schwartz[47]	eGFR = $(k \times Height)/Scr$[***]

(Scr): serum creatinine (mg/dL); **(BUN):** blood urea nitrogen concentrations (mg/dL); albumin (g/dL); **(F):** female; **(A.A.):** African-American. **Adopted by (NKF: K/DOQI, 2002).**

*This equation should be used when creatinine measurements have been calibrated to be traceable to IDMS.

**(K) is 0.7 for females and 0.9 for males; (a) is -0.329 for females and -0.411 for males; (min): minimum of Scr/K or 1; and max: maximum of Scr/K or 1.

***(k) is 0.33 for pre-term infant, 0.45 for full term infant and 0.55 for children of age 1-12.

However, creatinine is not a perfect GFR marker. Its production is affected by age, gender, ethnicity, and nutritional status **(NKF: K/DOQI, 2002).**

The estimated creatinine-based GFR, especially when the true GFR is greater than (60 mL/min/1.73m^2), can lead to the over diagnosis of CKD **(Hemmelgarn et al., 2010 and Glassock, 2010).** Despite these issues, it is still the most commonly used biomarker to assess GFR. However, there is a need for better GFR biomarkers **(Levey et al., 1999).**

2.3.3.2 Cystatin C

Cystatins C (Cys-C) are single chain proteins that reversibly inhibit cysteine proteinases belonging to the papain and legumain families **(Janowski et al., 2001).** The human cystatins form three groups based on molecular organization. Family 1 cystatins are found primarily intracellularly, without disulfide bonds and no carbohydrate side chains, there are two human representatives, cystatin A and cystatin B. Family 2 cystatins are mainly extracellular, contain two disulfide bridges. In humans, eight members of the cystatin family have been identified: cystatin C, D, E/M, F, G, S, SN and SA. Family 3 cystatins are multidomain proteins. These proteins are of quite high molecular mass, contain disulfide bonds and are glycosylated. The human representatives of this group are the kininogens

(Abrahamson et al., 2006), The human cystatin family presently comprises of 11 identified proteins. Two of these, cystatin A and B, form the family 1 Cystatin that are mainly intracellular proteins, while cystatin C, D, E, F, S, SA and SN constitute the family 2 Cystatins and are extracellular and/or transcellular proteins. The family 3 Cystatins, high and low molecular weight kininogen, are intravascular proteins, which in addition to being inhibitors of cysteine proteases also are involved in the coagulation process and in the production of vasoactive peptides **(Abrahamson et al., 2003)**. In general, cysteine proteinases are involved in the intracellular catabolism of peptides and proteins, processing of proenzymes and prohormones, breakdown of collagen, and bone resorption. The activities of the cysteine proteinases are controlled by naturally occurring inhibitory proteins such as α2-macroglobulin and cystatins **(Bobek and Levine, 1992)**.

Table (2.10): The human cystatin superfamily

Family 1	Family 2	Family 3
Intracellular cystatins	Extracellular and/or transcellular cystatins	Intravascular cystatins
Cystatin A	Cystatin C	LMW-kininogen
Cystatin B	Cystatin D	HMW-kininogen
	Cystatin E	
	Cystatin F	
	Cystatin G	
	Cystatin S	
	Cystatin SA	
	Cystatin SN	

Adopted by (Filler et al., 2005).

Cys-C is a cationic, low molecular weight cysteine protease inhibitor encoded by the Cystatin 3 (CST3) gene. It is ubiquitously expressed at moderate levels. Cys-C freely filtered at the glomerulus and completely reabsorbed and catabolized by tubular cells. it is an important extra- and transcellular inhibitor and its monomeric form is present in all human body fluids. It is especially abundant in cerebrospinal fluid, seminal fluid, milk, synovial fluid, saliva, tears, urine, and blood plasma **(Mahajan et al., 2016).**

Cys-C is a cysteine proteinase inhibitor belonging to the type 2 cystatin gene family **(Abrahamson et al., 1990 and Mussap & Plebani, 2004).** It is molecular

weight of 13.3 kDa (**Grubb and Lofberg, 1985.; Grubb, 1992.; Grubb, 2001.; Prigent, 2008 and Yassine et al., 2016**), and consists of 120 amino acids residues in a single polypeptide chain with two disulfide bonds and is produced by all nucleated cells (**Finney et al., 2000 and Filler et al., 2005**). Cys-C is a non-glycosylated single chain protein and produced at a constant rate by all nucleated cells (**Grubb, 1992 and Yassine et al., 2016**). and its production rate is unaltered in inflammatory conditions (**Simonson et al., 1985 and Grubb, 1992**).it is also freely filtered by the renal glomerulus membrane (**Tenstad et al., 1996 and Mahajan et al., 2016**). its reabsorbed and metabolized in the proximal tubule (**Levey et al., 1989**). Therefore, it has been used to monitor the progression of CKD (**Yassine et al., 2016**), and has been proposed as a suitable marker for GFR (**Simonson et al., 1985.; Grubb, 1992.; Dharnidharka et al., 2002.; Suri et al., 2004 and Domingueti et al., 2016**).

Structural analysis of the Cys-C gene and its promoter has shown that the gene is of the house-keeping type, which is compatible with a stable production rate by most cells, even under inflammatory stimuli (**Simonsen et al., 1985.; Grubb et al., 1985 and Abrahamson et al., 1990**). There are several properties that make Cys-C as a good candidate marker of GFR, including a constant production rate regulated by a "housekeeping" gene expressed in all nucleated cells, free filtration at the glomerulus, complete reabsorption and catabolism by the proximal tubules with no reabsorption into the bloodstream, and no renal tubular secretion .The functions of Cys-C is include involvement in extracellular proteolysis, modulation of the immune system, and antibacterial and antiviral activities (**Carlton, 2005**).

Cys-C, formerly known as γ-trace or post-γ- globulin, is a cysteine proteinase inhibitor that belongs to family 2 of the cystatin superfamily (**Filler et al., 2005**), and is a potent inhibitor of lysosomal cathepsins B, H, and L (**Janowski et al., 2001**). There have been several papers during recent years suggesting that Cys C measurement in serum correlates with GFR (**Simonsen et al., 1985.; Grubb et al., 1985 and Grubb, 1992**), and has recently been described as a promising endogenous marker of GFR both in adults (**Newman et al., 1995**), and in children (**Filler et al., 1997.; Bokenkamp et al., 1998 and Helin et al., 1998**). In contrast to serum creatinine and Cys-C is independent of height and body composition (**Bokenkamp et al., 1998**), it does not depend on muscle mass, sex and age (**Coll et al., 2000 and**

Sharma et al., 2009). Cys-C is a more sensitive indicator of mild renal impairment and better estimates the GFR than serum creatinine (**Khyse et al., 1994.; Le et al., 1999.; Donadio et al., 2001.; Dharnidharka et al., 2002 and Sarnak et al., 2005**), because, unlike creatinine, it is not secreted by the renal tubule, is not affected by muscle mass, and does not suffer the same problems with analytical interference (**Khyse et al., 1994).** These potential advantages have been reflected in several clinical studies, where measurement of Cys-C in patients with varying degrees of renal impairment has been shown to correlate more closely than serum creatinine to the Cr-labeled EDTA determination of GFR (**Nilsson et al., 1994 and Newman et al., 1995**). Cys-C, a reliable marker of renal function, and used to estimate GFR. It is not only associated with renal disease (**Domingueti et al., 2016**), but also related to obesity (**Luc et al., 2006**), diabetes mellitus (**Pucci et al., 2007**), metabolic syndrome (**Al Wakeel et al., 2008**) and thyroid disorders (**Krishna et al., 2012**). Because of its independence from age and gender (**Dharnidharka et al., 2002).** It is hypothesized that serum cys-C levels are influenced by the method and intensity of dialysis received (**Suri et al., 2004).** Dialysis efficiency greatly influences the well-being, out come and survival of patients with CKD. Presently, the efficacy of dialysis is assessed by estimating serum creatinine and serum Cys-C (**Van Den et al., 2002**). Use of serum Cys-C value (or its reciprocal) as a measure of GFR was proposed in 1985 (**Grubb et al., 1985 and Simonsen et al., 1985).** Since that time, multiple studies have been performed to evaluate the accuracy of Cys-C level as a marker of GFR (**Newman et al., 1995.; Risch et al., 1999 and Woitas et al., 2000**). The crystal structure of human Cys-C composed of five-stranded antiparallel β-sheets partially wrapped around a central α- helix (**Figure:2.4) (Philippe, 2008**)

Figure (2.4): Crystal structure of human cystatin C (RCSB /PDB,2016)

Sources: http://cutt.us/mjKL1

The presence of a hydrophobic leader sequence in pre-cys-C precursor strongly indicates that the protein is normally secreted. Cys-C is widely distributed in body fluids such as cerebrospinal fluid (CSF), seminal fluid, saliva, blood plasma, and urine. It is also present in tissues, including brain, kidney, liver, placenta, and seminal vesicles **(Filler et al., 2005)**.

2.3.3.2.1 Determination of cystatin C

Cys-C is commonly determined in biological material via three methods. ELISA – has the advantage of measuring low concentrations. Its disadvantage is the impossibility of statim testing. Nephelometry and turbidimetry can be performed statim but in this case, low concentrations cannot be tested. The disadvantages of turbidimetry are its low robustness and low calibration stability. Nephelometry is considered the best technique in this area **(Mares et al., 2003),** a number of automated methods have been developed to measure Cys-C, including a commercially available Particle Enhanced Turbidimetric Immunoassay (PETIA) **(Kyhse et al., 1194),** an in-house latex (PETIA) **(Newman et al., 1995),** and a latex particle enhanced nephelometric immunoassay (PENIA). The majority of these methods use technologies that are applicable to the same instruments with which serum creatinine is analyzed **(Finney et al., 1997)**.

2.3.3.2.2 Serum cystatin C values:

The mean concentration of serum Cys-C values of all subjects rose significantly between ages in healthy adult individual's ranges between (0.8-1.2 mg/L) depending upon the analytical method used **(Finney et al., 2000 and Bashir et al., 2010).**

2.3.3.2.3 Cytogenetic and molecular location of CST3 gene

The CST3 gene provides instructions for making a protein called Cys-C. This protein is part of a family of proteins called cysteine protease inhibitors that help control several types of chemical reactions by blocking (inhibiting) the activity of certain enzymes. Cys-C inhibits the activity of enzymes called cathepsins that cut apart other proteins in order to break them down **(Genetic Home Reference, 2016).** The cytogenetic location of cystatin is: 20p11.21, which is the short (p) arm of chromosome 20 at position 11.21, and its molecular location: base pairs 23,627,897

to 23,638,048 on chromosome 20, Other names for this gene cystatin-3, cys-C, cys-C precursor, CYTC_HUMAN, gamma-trace, neuroendocrine basic polypeptide and post-gamma-globulin. **(Genetic Home Reference, 2016). (Figure: 2.5)**

Figure (2.5): Cytogenetic location of cystatin

Adopted from http://cutt.us/2taVa (Genetic Home Reference,2016)

2.4 Hemodialysis and peritoneal dialysis

Dialysis modality selection for ESRD patients three primary treatment options for are HD, PD and kidney transplantation **(United States Renal Data System, USRDS, Annual Data Report, 2009).** The dialysis uses very basic concepts, such as osmosis and diffusion, to clear extra fluid and substances from the body. The first person to describe this process was *Thomas Graham*, known as the 'Father of Dialysis'. He first studied diffusion in gases and later performed a series of experiments in liquids **(Graham, 1854)**, He predicted that 'dialysis' would be an important treatment for renal failure. the blood is passed, a little at a time, through a special filter, which removes waste products and excess fluid. The purified blood is then returned to the body. The process is done outside of the body through a hemodialysis machine and a dialyzer. A strict schedule consisting of sessions of 3 to 5 hours, on alternate days, 3 days a week is usually followed **(FHN Trial Group et al., 2010).**

The number of patients being treated for ESRD globally was estimated to be 2,786,000 at the end of 2011 and, with a 6-7% growth rate, continues to increase at a significantly higher rate than the world population. Of these 2,786,000 ESRD patients, approximately 2,164,000 were undergoing dialysis treatment HD or PD and around 622,000 people were living with kidney transplants. In the USA, Japan and the European Union, dialysis patient population growth rates between 2010 and 2011 were in a range of 1– 4% and, as such, were significantly lower than growth rates in regions such as Asia, Latin America, the Middle East and Africa **(Fresenius Medical Care report, 2011).**

In Palestine renal failure is one of the most important problems on the healthcare delivery system. As per the year 2000 and 2001 statistics, there were 351 and 400 patients who were maintained on HD and PD, respectively. The most common causes for ESRD in Palestine is glomerulonephritis and diabetic nephropathies. The death rate among patients on dialysis is 7-8%; the cardiac and cerebrovascular complications are the main causes of death. The HD services in Palestine were initiated in 1972 **(Shahla, 2003),** there are 12 working HD centers in Palestine, 8 in West-Bank and 4 in Gaza **(Hemodialysis Services, 2016).** The Palestinian Health Annual Report (2010) showed that renal failure constitutes one of the ten leading causes of death in the Gaza strip with mortality rate of 2.8% **(Ministry of Health, MOH, 2010).**

The NKF, K/DOQI recommends that planning for dialysis begin when patients reach CKD stage 4 (eGFR or creatinine clearance [CL_{cr}] below 30 mL/min per 1.73 m^2 [0.29 mL/s/m^2]) **(NKF: K/DOQI, 2006).** Beginning the preparation process at this point allows adequate time for proper education of the patient and family and for the creation of a suitable vascular or peritoneal access. For patients choosing HD, a permanent arteriovenous (AV) access (preferably a fistula) should be surgically created when Cl_{cr} or eGFR falls below 25 mL/min (0.42 mL/s), serum creatinine is greater than 4 mg/dL (354 μmol/L) or 1 year prior to the anticipated need for dialysis **(Himmelfarb et al., 2008).**

2.4.1 Hemodialysis

Although HD was first successfully used in 1940, the procedure was not used widely until the Korean War in 1952. Permanent dialysis access was developed in the 1960s, which allowed routine use of HD in patients with ESRD. Subsequent decades brought advances in dialysis technology, including the introduction of more efficient and biocompatible dialyzer membranes and safer techniques. HD is now the most common type of renal replacement therapy for patients with ESRD **(Brescia et al., 1999 and Quinton et al., 2004).**

Both of PD and HD use the concepts of diffusion and convection clearance. PD uses the peritoneal blood flow, which allows molecules and fluid exchange to occur between the blood and dialysate in the peritoneal cavity. The dialysate fluid is

drained and then the new dialysate fluid is infused. It is considered a continuous dialysis therapy. Similarly, HD uses the diffusion and convection clearance mechanisms. However, the blood is first removed from the body. As a result, fluid and molecules are exchanged outside of the body through a hemodialysis machine and a dialyzer. Dialysate runs in the counter-current direction in the dialyzer to maximize the concentration gradient. The 'clean' blood is then returned back to the patients. The therapy is usually performed three times per week, with four hours each session. However, prolonged and/or frequent hemodialysis is performed only in a small population of patients, usually in the home setting **(DiPiro et al., 2011).**

HD is a method that is used to achieve the extracorporeal removal of waste products such as creatinine and urea and free water from the blood by an artificial kidney machine when the kidneys are in a state of renal failure is frequently done to treat ESRD. The basic principle of the artificial kidney is to pass blood through minute blood channels bounded by a thin membrane. On the other side of the membrane is a dialyzing fluid into which unwanted substances in the blood pass by diffusion **(Guyton & Hall, 2011).** HD, simply stated, consists of the perfusion of blood and a physiologic solution on opposite sides of a semipermeable membrane. Multiple substances, such as water, urea, creatinine, uremic toxins, and drugs, move from the blood into the dialysate, by either passive diffusion or convection as the result of ultrafiltration. The ultrafiltration is the movement of water across the dialyzer membrane as a consequence of hydrostatic or osmotic pressure and is the primary means for removal of excess body water **(DiPiro et al., 2011).**

HD is found in two variants: conventional HD, where patients receive HD in a clinic three times a week for 4 hours/session, and nocturnal HD, where patients are trained to do their own HD while they sleep, 5–6 nights/week **(Crawford and Lerma, 2008).** HD, the three basic types of vascular access are necessary, The Arteriovenous (AV) fistula, Arteriovenous (AV) graft, or central venous catheter through which blood is obtained for dialysis is referred to as the dialysis access **(Hayashi et al., 2006 and DiPiro et al., 2011). (Figure: 2.6)**.

Figure (2.6): The predominant types of vascular access (A) the arteriovenous (AV) fistula and (B) the synthetic arteriovenous forearm graft.

Adopted from (DiPiro et al., 2011).

The five major components of HD are: a vascular access, a dialysate circuit, a blood circuit, a dialyzer and a HD machine. And There are three broad categories of dialysis membranes: conventional or standard (low- flux), high efficiency, and high-flux. In HD, the patient's blood is pumped to the dialyzer at a rate of 300 to 600 mL/min **(Ahmad et al., 2007).** The therapy is usually performed three times per week, for 3-5 hours each session, this is a substantial time commitment for patients undergoing HD and results in substantial loss of control over their life **(Annual Report, ESRD-CPMP, 2006).** Other types of HD have been explored in an effort to balance dialysis adequacy with patient outcomes and quality of life. Quotidian dialysis is a variant of HD in which dialysis is administered daily for shorter periods of time (2–2.5 hours) or as long, slow nocturnal treatments of up to 6 to 8 hours **(Pierratos et al., 2005 and Pierratos, 2008).**

HD is a relatively safe procedure but there are several Complications associated with HD therapy are significant and can limit therapy efficacy. These complications, which occur during the actual therapy (intradialytic), as well as those associated with vascular access. The most common complications that occur during the HD procedure include hypotension, cramps, nausea and vomiting, cardiac arrhythmias, anaphylaxis, headache, Pruritus, chest pain, back pain, restless leg syndrome and fever or chills, these complications and their etiology and predisposing factors. **(Sarkar et al., 2005.; Sherman et al., 2007 and Crawford & Lerma, 2008).** However, with proper monitoring and prompt treatment, many of these complications can be avoided. Of note, better glycemic control (HbA1c < 7.5 %) has been shown to predict better survival of diabetic ESRD patients starting HD treatment **(Morioka et al., 2001). (Figure: 2.7)**.

Figure (2.7): A simple schematic diagram of hemodialysis machines and dialyzer Adopted from (DiPiro et al., 2011).

2.4.1.1 Advantages and disadvantages of hemodialysis

Advantages	Disadvantages
• Higher solute clearance allows intermittent treatment. • Parameters of adequacy of dialysis are better defined and therefore under dialysis can be detected early. • Technique failure rate is low. • Even though intermittent heparinization is required, hemostasis parameters are better corrected with HD than with PD. • In-center HD enables closer monitoring of the patient.	• Requires multiple visits each week to the HD center, which translates into loss of patient independence. • Disequilibrium, dialysis-induced hypotension, and muscle cramps are common. may require months before the patient adjusts to HD. • Infections in HD patients may be related to the choice of membranes, the complement-activating membranes being more deleterious. • Vascular access is frequently associated with infection and thrombosis. • Decline of residual renal function is more rapid compared with peritoneal dialysis.

Adopted from: (Sherman et al., 2007 and Pierratos, 2008).

2.4.2 Peritoneal dialysis

The concept of peritoneal lavage has been described as far back as the 1700s, it wasn't until the 1920s that PD was first employed as an acute treatment for uremia. It was used infrequently during subsequent years until the concept of PD as a chronic therapy for ESRD was proposed in the 1960s; by the mid-1970s it was used relatively commonly. Over the ensuing years the number of patients receiving PD increased slowly until the early 1980s. At that time, several innovations in PD delivery systems were introduced. These innovations led to improved outcomes, decreased morbidity, and a corresponding increase in the use of PD as a viable alternative to HD for the treatment of ESRD, However, even with these proposed

advantages, there has been a declining use of PD in the world over the past decade **(Khawar et al., 2007 and DiPiro et al., 2011).**

PD uses the peritoneal blood flow, which allows molecules and fluid exchange to occur between the blood and dialysate in the peritoneal cavity and lining (peritoneum). The dialysate fluid is drained and then the new dialysate fluid is infused. It is considered a continuous dialysis therapy. Compared to HD, PD offers lower risk of death across all subgroups for the first 1–2 years of dialysis and is now recommended for use as the initial modality of dialysis in the majority of ESRD patients due to the lower prevalence of infections and better preservation of residual renal function **(Chung et al., 2009 and DiPiro et al., 2011).** The three basic components of are also present in PD a blood-filled compartment separated from a dialysate-filled compartment by a semipermeable membrane **(Sharma and Blake, 2008).** The two common choices types for PD are **continuous ambulatory peritoneal dialysis(CAPD).** it is where several exchanges may be done during the day manually called the mini-cycler, and automated **continuous cycling peritoneal dialysis(CCPD)** it is where the exchange is done automatically by the machine while the patient sleeps. the CCPD is more common than CAPD, both of which function by infusing peritoneal dialysis fluid in the peritoneal cavity and draining it 4–6 hours later with the number of exchanges varying according to patient size, the prescribed dose of PD may be altered by changing the number of exchanges per day, by altering the volume of each exchange, the machine performs several short-dwell exchanges (usually 1 to 2 hours) during the night. This permits a long cycle-free daytime dwell of up to 12 to 14 hours. the peritoneal membrane permeability, and residual kidney function **(Peritoneal, 2006.; Crawford & Lerma, 2008 and DiPiro et al., 2011).**

The complications of PD, mechanical, medical, and infectious problems, pain from impingement tip and kinking of the catheter , aggravation of tissues, increased adipose tissue deposition, decreased appetite, malnutrition, requirements for insulin in diabetic patients, fibrin formation in dialysate **,** infectious or peritonitis complications of PD are a major cause of morbidity and mortality and are the leading cause of technique failure and transfer from PD to HD. Fluid overload and electrolyte abnormalities **(DiPiro et al., 2011)**. **(Figure: 2.8)**.

Figure (2.8): Diagram of the placement of a peritoneal dialysis catheter through the abdominal wall into the peritoneal cavity

Adopted from (**DiPiro et al., 2011**).

2.4.2.1 Advantages and disadvantages of peritoneal dialysis

Advantages	Disadvantages
• Hemodynamic stability due to slow ultrafiltration rate. • Higher clearance of larger solutes, which may explain good clinical status in spite of lower urea clearance. • Better preservation of residual renal function. • Convenient intraperitoneal route for administration of drugs such as antibiotics and insulin. • Suitable for elderly and very young patients who may not tolerate HD well. • Freedom from the "machine" giving the patient a sense of independence (for continuous ambulatory peritoneal dialysis). • Less blood loss and iron deficiency, resulting in easier management of anemia or reduced requirements for erythropoietin and parenteral iron. • No systemic heparinization required. • Subcutaneous versus intravenous erythropoietin or darbepoetin is usual, which may reduce overall doses and be more physiologic.	• Protein and amino acid losses through peritoneum and reduced appetite owing to continuous glucose load and sense of abdominal fullness predispose to malnutrition. • Risk of peritonitis. • Catheter malfunction, exit site, and tunnel infection. • Inadequate ultrafiltration and solute dialysis in patients with a large body size, unless large volumes and frequent exchanges are employed. • Patient burnout and high rate of technique failure. • Risk of obesity with excessive glucose absorption. • Mechanical problems such as hernias, dialysate leaks, hemorrhoids, or back pain more common than with HD. • Extensive abdominal surgery may preclude PD. • No convenient access for intravenous iron administration.

Adopted from: (Sherman et al., 2007 and Pierratos, 2008).

2.4.3 Kidney transplantation

Is the surgical procedure of placing a fully functioning kidney into a person with ESRD, this procedure is usually an elective one, performed in patients who have undergone careful preoperative assessment and preparation. The transplanted kidney may originate from a deceased donor or from a related or unrelated person **(Cueto and Rojas, 2007).** Several recent studies have demonstrated significantly improved patient and allograft survival as well as lower rates of delayed graft function or acute rejection episodes in those with preemptive transplants versus those who were on dialysis for a period of time before transplantation **(Gill et al., 2004 and Baura, 2012).**

2.5 The physiology of hemodialysis clearance

Dialysis clearance is defined as the volume of blood from which all solutes in question is reduced during a specified time period. The total amount of solute removal depends on the duration of the therapy; this is similar to renal clearance in the healthy kidney. There are two type of clearance, diffusional clearance occurs when solutes from solution A (blood) move to solution B (dialysate) by concentration differences through a semipermeable membrane. molecules and ions dissolved in the solutions are in constant motion, the molecules on average moves from high concentration solution to low concentration solution, the ultrafiltration refers to the situation where water molecules move from solution A (blood) to solution B (dialysate). This process is driven by either a hydrostatic or an osmotic gradient through a semipermeable membrane. Convectional clearance can occur when water moves from one solution to another solution through the semipermeable membrane (i.e. blood to dialysate), and the water is accompanied by other solutes. A healthy person drinks ~1.5 – 2 L of water per day which is 10 -15 L per week, but a HD patient can be exposed to ~ 400 L of water per week **(Shih-Han, 2013).**

The duration and frequency of HD treatments can also affect a patient's total solute clearance, although conventional HD three times per week, four hours per session it is the current standard of care and highly efficient in removing small solutes, and it has a high clearance rate per session **(Gotch, 1998 and FHN Trial Group et al., 2010).**

2.5.1 Dialysis adequacy and outcomes

The optimistic goals, of dialysis outcomes patients and idealistic clinical perspective, an adequately treated HD patient is physically active, well nourished, euvolemic, and normotensive with a maintained good quality of life and a life expectancy that is not inferior to that of healthy patients. Dialysis efficiency greatly influences the wellbeing, outcome and survival of patients with ESRD. Therefore, a close monitoring and follow up is essential, so as to achieve the best outcome. The Mortality in HD patients differs by gender and race, with an annual mortality rate of 13% to 20%, the outcomes directly related to many factors occurring during evolution of CKD, such as atherosclerotic vascular disease, diabetes, and arterial hypertension, and to background CVD mortality of the respective general population **(Yoshino et al., 2006 and Johnson et al., 2015).**

Other major factors influencing outcome that are common to all patients with ESRD include anemia and control of bone and mineral metabolism, a key factor influencing outcome is "adequacy" of dialysis, in patients receiving maintenance HD and measures to influence these factors clinically, which was originally used exclusively to describe dialysis dosage measured by small solute removal, but now has a broader meaning encompassing all aspects of the replacement of excretory and endocrine functions of the kidney that affect outcome, these factors that influence HD solute clearance are grouped as dialysis-related, patient-related, and solute-related factors (**Johnson et al., 2015 and Sahutoglu et al., 2016).**

Table (2.11): Factors influencing and related to outcome of dialysis patients

Dialysis-related	Patient-related	Solute-related
Session length	Gender and Age	Intracellular Concentration
Frequency	Body weight and Fluid overload	Molecular weight
Long interdialytic interval	Social status	Dialysate quality
Dialysis dose	Residual renal function	Dialysate composition
Dialysis modality	Vascular access	Protein Binding
Hemodialysis membrane biocompatibility	Protein-energy malnutrition	Charge
Hemodialysis membrane type	Low serum albumin and Inflammation	
Blood flow	Intake and absorption	
Dialysate flow	Blood viscosity	
Ultrafiltration	Diabetes mellitus, hypertension, Congestive heart failure and Anemia	

Adopted from (Johnson et al., 2015 and Sahutoglu et al., 2016)

2.5.2 Adequacy of hemodialysis

The term "dialysis adequacy" has been expressed mostly to achieve the minimally acceptable Kt/V urea target, and has largely abandoned the importance of additional clinical measures among patients with ESRD. Indeed, the totality of dialysis adequacy should reflect measures that comprehensively aim to maximize the sum of survival, quality of life, cardiovascular outcomes, and other patient-related outcomes (**Perl et al., 2017**). The rising trend of CKD and absence of adequate dialysis are the main causes of death in kidney patients. Thus, determining dialysis adequacy in HD patients can help to develop better healthcare (**Barzegar et al., 2016**).

The effective HD treatment, decreases the mortality in patients with kidney disease, and can reduce the rates of complications (**Barzegar et al., 2016 and Nemati, 2017).** By improving the dialysis adequacy, uremic complications and their effects on different organs will be reduced. Therefore, increasing dialysis quality is effective on various aspects of life in patients with CRF (**Barzegar et al., 2016**). According to the conducted study by national cooperative dialysis study (NCDS) as much as the efficiency of dialysis is higher, complications of uremia in body organs are reduced (**Nemati, 2017).**

According to the findings of previous studies, dialysis adequacy is a predictor for mortality and morbidity in patients undergoing HD, and patients undergoing adequate dialysis have a lifespan equal to that of patients with renal transplant (**Saad et al., 2015**). On the other hand, inadequate HD increases the patient's need for more prolonged or frequent hemodialysis, reduces the quality of life, imposes additional treatment costs on the national health system (**Biniaz et al., 2018**).

2.5.3 Adequacy of dialysis dose

2.5.3.1 Uremic toxins

The uremic syndrome is attributable to the progressive retention of a large number of compounds that normally are metabolized by healthy kidneys or filtered and secreted into the urine it is called uremic retention solutes or uremic toxins when they interact negatively with biologic functions (**Maheshwari et al., 2016).** The Uremic toxins include a small group of inorganic compounds as small plasma

solutes, such as water, potassium, phosphate, and trace elements, and a much larger group of organic compounds that are further subdivided into small water-soluble solutes it is low-molecular-weight solutes (<500 Da). such as (creatinine, uric acid, urea) and protein-bound solutes such as (homocysteine) and middle molecules (MM) (>500 Da) such as (Cytokines, ANP, β2-microglobulin, Cys-C, Hyaluronic acid, Leptin, and PTH) (**Maheshwari et al., 2016 and Johnson et al., 2015**).

The conventional low flux (LF) dialyzer permits effective small solute clearance, but its clearance of middle molecules is relatively lower. High flux (HF) dialyzer allows more efficient removal of small water- soluble uremic compounds as well as middle molecules and ensures improved dialysis quality and reduces the short-.and long-term hemodialysis-related complications (**Maheshwari et al., 2016**).

The mortality has associated with reduced clearance of urea, creatinine and commonly of middle molecular size substances. Although it is evident that uremic toxicity is more than the retention of urea or water soluble compounds, while current dialysis does not remove any significant quantity of substances with higher-molecular weight toxins or protein-bound substances (**Johnson et al., 2015**).

2.5.3.2 Marker used to assess dialysis clearance

The potential of uremic toxins these are uremia toxicity related to the retention of organic waste solutes it was fatal before dialysis became available, urea is established as a marker of dialysis clearance and uremic solute retention and removal., it is generation therefore depends on protein intake and the balance between protein anabolism and catabolism and show as a little toxicity, the removal of urea was considered to be representative for the removal of other water-soluble solutes with a higher pathogenic impact, but it is now clear that urea removal does not closely parallel that of other small water-soluble compounds, protein-bound solutes and middle molecules compounds (**Vanholder et al., 2003 .; Duranton et al., 2012 and Johnson et al., 2015**). The use of serum urea is recommended by the kidney disease outcome quality improvement clinical practice guideline to assess dialysis clearance (**Levin, 2006**).

In addition to urea as a marker of dialysis clearance it found some interest marker for dialysis clearance or efficacy is assessed by estimating serum creatinine

levels before and after each session of dialysis, however serum creatinine levels are influenced by inter-individual variability related to age and gender and also affected by body composition, dietary factors, Numerous drugs and endogenous substances such as ketone bodies (**Krishnamurthy et al., 2010**).

The URR and CrRR that are commonly used can assess the removal of only small solutes or dialysis adequacy by conventional HD (**Maheshwari et al., 2016**). Currently the determination of serum Cys-C has been proposed as an additional parameter for assessment of renal function (**Dharnidharka et al., 2002**). Several studies have suggested that Cys-C is useful as a marker of HD toxin removal, since it has the attractive features as a representative middle molecule (**Campo et al., 2004**). Though assess to Cys-C reduction ratio (CCRR) can be used as an alternative indicator of middle molecule clearance or dialysis adequacy (**Maheshwari et al., 2016**).

2.5.3.3 Assessment of hemodialysis adequacy

Low efficiency of HD increases the need for more HD sessions, longer hospitalizations, and increased hospital costs. Therefore, monthly assessment of HD adequacy is recommended and can be assessed in several ways (**Rezaiee et al., 2016 and Shanthala et al., 2016**). According to NKF: DOQI guidelines, the most common acceptable methods to quantify the dose of dialysis are UKM, URR, natural kt/v and the daugirdas second generation formula which also called Sp-Kt/V (**Breitsameter et al., 2012 and Shanthala et al., 2016**). Moreover, CrRR and CCRR are also used to quantify the dose and adequacy of dialysis. (**Levin, 2006; Johnson et al., 2015 and Maheshwari et al., 2016**).

Minimum sp-Kt/v and the urea reduction rate are the indicators of dialysis adequacy. According to the kidney diseases outcomes quality (KDOQI) guidelines for HD patients, the minimally adequate dose of dialysis should be a sp-Kt/v of 1.2 or URR of 65% (**Gilmore, 2006 and Baral et al., 2017**).

2.5.3.3.1 Urea reduction ratio

URR is a measure of adequacy of delivered dose of dialysis expressed as a percentage reduction in blood urea level after a session of dialysis which is mathematically related to Kt/V (**Chijioke et al., 2016**). It refers to the reduction in

serum urea concentration during dialysis treatment (**Kotanko et al., 2008**), and it used to describe the change in urea concentrations. The URR correlates well with dialysis outcome and is an accepted method for assessment of dialysis adequacy. (**CPGHA, 2006 and Johnson et al., 2015**). URR is mathematically related to sp-Kt/V (**Kotanko et al., 2008**).

URR was first popularized by **Lowrie** and **Lew** in 1991 as a method of measuring amount of dialysis that correlated with patient outcome (**Shanthala et al., 2016**). URR is recognized by the KDOQI guidelines as an acceptable method to quantify the dialysis dose (**KDOQI, 2006 and Kuhlmann et al., 2010**). URR is the simplest and most commonly used parameter to express dialysis, It's a comparison of the pre- and post-dialytic serum urea concentrations (**Kuhlmann et al., 2010; Johnson et al., 2015; Shanthala et al., 2016 and Mohamed et al., 2018**).

Although Kt/V is recommended as the best measure of dialysis adequacy, URR is the most utilized because of its simplicity with both methods having similar predictive power in terms of patient outcome (**Kotanko et al., 2008 and Chijioke et al., 2016**). In contrast to other methods, the URR method does not take into account that urea is additionally removed from the blood by ultrafiltration (**KDOQI, 2006.; Kuhlmann et al., 2010 and Mohamed et al., 2018**). As such, the greater the ultrafiltration volume removed during dialysis, the more inaccurate the results of dialysis dose calculation become based on URR. As such, most centers use Kt/V, which incorporates ultra-filtrate urea losses. (**Mohamed and Davenport, 2018**). The efficacy of dialysis was assessed by calculating the reduction ratio for serum Urea, creatinine and Cys-C is computed as follows:

$$URR\% = 100 \, X \left(1 - \frac{C_t}{C_0} \right)$$

Where C_t and C_o represent the post-dialysis and pre-dialysis serum urea concentrations, respectively (**Kotanko et al., 2008; Krishnamurthy et al., 2010 and Johnson et al., 2015**).

In order to provide adequate clearance, the KDOQI guidelines recommend that hemodialysis treatments less than five hours should have a minimum URR of 65% with a target dose of 70% (**KDOQI, 2006**). According to KDOQI guidelines, URR

equivalent to 65% is considered the minimum standard criteria for adequate HD, URR of 65% and higher is considered optimal HD adequacy, URR of 55–64. 99% is considered relatively favorable, and URR of less than 55% is considered undesirable HD. Moreover, a URR of less than 65% is associated with increased morbidity and mortality among patients **(KDOQI, 2006 and Rezaiee et al., 2016).** It has been shown that for every 5% increase in URR, mortality rate decreased up to 11%. **(Shariati et al., 2012 and Rezaiee et al., 2016).**

2.5.3.3.2 Kt/V index:

Kt/V is the fractional urea clearance, defined as urea clearance (K) multiplied by the dialysis session length (t) divided by the urea distribution volume (V). Kt/V is of clinical interest, as it has been shown to correlate with morbidity in the national cooperative dialysis study **(Daugirdas, 1993)**. The concept behind Kt/V arose from a reanalysis of the national cooperative dialysis study (NCDS) by Gotch and Sargent in 1985. The subsequent widespread adoption of Kt/V effectively simplified the concept of adequacy to the achievement of small solute clearance **(Jones et al., 2018).** Kt/V the better method for measuring dialysis dose because it takes account of the size of a patient and urea removal by ultrafiltration **(Pyart et al., 2018).** Assuming no ultrafiltration or urea generation, the delivered Kt/V can be calculated from the urea concentration at the start and end of dialysis using the formula below **(Locatelli et al., 2004).**

$$Kt/V = ln\left(\frac{C_0}{C_t}\right) \qquad \text{or} \qquad Kt/V = -Ln\,(1 - URR)$$

Where *(ln)* stands for the natural logarithm, (C_o) is the initial urea concentration, and (C_t) is the ending urea concentration.

In the Kt/V ratio, the dialyzer urea clearance (K) is multiplied by dialysis time (t), the product being then divided by the patient`s urea distribution volume (V). (K) depends on dialyzer size, blood flow rate and dialysate flow. Although t normally ranges between 3 and 4 hours (180-240 minutes per dialysis session), it can be adjusted. The patient`s urea distribution volume (V) corresponds to approximately

50% of body weight, and may be more precisely estimated with an anthropometric equation which considers gender, age, height and weight **(Breitsameter et al., 2012).**

Each 0.1 decrease in Kt/V is associated with approximately 7% increase in the relative risk of death and 11% increase in the annual rates of hospitalization **(Amini et al., 2011)**. High Kt/V is one of the main objectives of HD and has a significant effect on the prognosis of patients undergoing dialysis; therefore, the factors affecting it must be carefully controlled and monitored. **(Abedi-Samakoosh et al., 2018).** The minimal prescribed Kt/V value was 1.2, (A value of 1.2 or higher is widely considered to indicate adequate HD in patients with ESRD. **(Schiffl et al., 2002; and Johnson et al., 2015)**. Kt/V of less than 0.8 is considered as a sign of inadequacy**,** and shown to be especially undesirable **(Daugirdas, 1993; Abedi-Samakoosh et al., 2018).** Because Kt/V is tightly linked to the post- serum divide on pre -serum urea ratio (R), or the percent urea reduction, a variety of formulas have been proposed as bedside estimates of the delivered Kt/V. One such estimate has been Kt/V = -In (R) **(Daugirdas, 1993).** Unfortunately, such a simple equation cannot account for other factors that may affect the delivered dose of dialysis **(Locatelli et al., 2004 and O'Connor et al., 2009).**

The final concentration of urea not only depends on urea removal by the dialyzer, but also on urea generation (G) and the convective effects of ultrafiltration. Similarly, the volume of distribution for urea (V) is not fixed and will vary according to intradialytic water removal., As such, UKM (sometimes called formal UKM) was developed as a more accurate method for determining Kt/V **(Gotch, 2001.; Locatelli et al., 2004 and Depner, 2009).** These models simulate the movement of urea during the dialysis session and derive values for V and G to calculate the dialysis dose **(Kemp et al., 2001 and Locatelli et al., 2004).**

2.5.3.3.3 Single-Pool Kt/V

Sp-Kt/V is the most popular method of determination of HD adequacy, assessing a single HD clearance **(Johnson et al., 2015).** sp-Kt/V is also the most common model for calculating Kt/V, it's based on the assumption that urea is located in only one compartment (or pool) of the body **(Daugirdas 1993; O'Connor, et al., 2009 and Kuhlmann, et al., 2010).** The concept of Kt/V may be applied to any

substance, and used almost exclusively for urea (**Schiffl et al., 2002 and Johnson et al., 2015**). The sp-Kt/v is a numerical value obtained by formula using variable like pre and post-dialysis urea or blood urea nitrogen, post-dialysis weight and ultra-filtrate volume (**Baral et al., 2017**).

The idea of sp-Kt/V predicts a linear decline in urea and an immediate equilibration between the blood and tissue compartments after dialysis. Thus, the sp-Kt/V is calculated through measurement of the pre-dialysis urea concentration, followed by the post-dialysis urea concentration 10-15 seconds after the end of dialysis (**Gotch et al., 2000 and KDOQI, 2006).**

Currently, sp-Kt/v is the only randomized control trial validated outcome measure for adult thrice-weekly HD is sp-Kt/V. All other methods of estimating dialysis dose are based on mathematical modelling from the underlying sp-Kt/V values, and any outcomes based on these metrics, e.g. Equilibrated Kt/V (eKt/V), are assumed to map directly back to the underlying spKt/V values from which they were created. Unfortunately, sp-Kt/V overestimates the true dialysis dose delivered by failing to account for post-dialysis urea rebound (**Mammen et al., 2010**).

Recent methods used in clinical practice sp-Kt/V may be computed according to the classic daugirdas equation, which is based on URR and accounts for intradialytic urea generation and ultrafiltration volume. is widely used because of its simplicity and accuracy in the Post-dialysis blood urea levels of the same dialysis session were measured to calculate the delivered dose of dialysis by using formula below:

$$SpKt/V = -Ln\,(R - 0.008 \times t) + (4 - 3.5 \times R) \times \frac{UF}{W}$$

where **K** is the dialyzer blood water urea clearance (liters per hour or milliliters per minute), **t** is dialysis session length (hours or minutes), **V** is the distribution volume of urea within the patient in (liters or milliliters, **ln** is the natural logarithm, **R** is the postdialysis-predialysis serum urea ratio or [predialysis (BUN) - postdialysis BUN/predialysis BUN], **t** is treatment time (hours), **UF** is ultrafiltration volume (liters), and **W** is the patient's postdialysis body weight (kilograms) (**Daugirdas, 1993; Schiffl et al., 2002; Daugirdas, 2007; Kuhlmann, et al., 2010; Johnson et al., 2015; Sahutoglu et al., 2016 and Baral et al., 2017).**

The sp-Kt/V equation is an example of a simplified, second generation logarithmic UKM formula used to calculate sp-Kt/V (**Daugirdas, 1993; Kuhlmann, et al., 2010 and Baral et al., 2017**). However, it should be noted that this equation is only accurate when applied to dialysis given thrice-weekly for 2.5-5 hours. The HEMO Study showed that the minimum dose established by the KDOQI guidelines is appropriate when dialysis is performed three times per week for 2.5 to 4.5 hours. (**KDOQI, 2006**). The Daugirdas equation is validated for a Kt/V range of 0.8 to 2.0 (**Johnson et al., 2015**), but The NKF-KDOQI clinical practice guideline for hemodialysis adequacy:2015 update recommends the minimally adequate dose for conventional is spKt/V of 1.2, with a target dose of 1.44 per hemodialysis session for patients treated thrice weekly (**KDOQI, 2006; Mammen et al., 2010 and Rocco et al., 2015**).

2.5.3.3.4 Cystatin C and creatinine reduction ratios

CrRR are commonly used to assess the removal of only small solutes by conventional HD (**Maheshwari et al., 2015**). Several studies have suggested that cys-C is useful as a marker of HD toxin removal., Since, cys-C has the attractive features as a representative MM, and it could be applied to measure MM clearance when it represented in the form of CCRR (**Thysell et al., 1988; Campo et al., 2004 and Maheshwari et al., 2015**).

The removal of cys-C in a single HD treatment, CCRR depends on normalized blood liters processed and fluid removal during HD (**Huang et al., 2011**). Both CrRR and CCRR are used though HF dialyzers membranes to assess the dialysis adequacy (**Maheshwari et al., 2015**). A recent study showed that the cys-C clearance with HF hemodialysis was more effective compared with LF hemodialysis, the variables affecting CCRR were not identified (**Park et al., 2010**). Efficacy of dialysis was assessed by calculating the reduction ratio for serum creatinine as shown below:

$$\boldsymbol{CrRR \ and \ CCRR = 100 \ X \left(1 - \frac{C_t}{C_0} \right)}$$

where **C_t** & **C_0** represent post-dialysis and pre-dialysis serum creatinine levels.
The same formula was also used for the calculation of serum CCRR (**Krishnamurthy et al., 2010 and Maheshwari et al., 2015**).

2.6 Previous studies

In Barcelona, Spain, Coll et al., (2000). conducted a study to compare serum cys-C to serum creatinine level and creatinine clearance as markers of GFR and to analyze their changes according to renal failure, cys-C determined by iothalamate labeled with iodine 125 (^{125}I-iothalamate; Amersham Laboratories, Buckinghamshire, England) clearance (^{125}I-ICl) values. The concentrations of the two different markers of GFR in patients with impaired renal function were classified according to ^{125}I-ICl. Twenty individuals with normal renal function (^{125}I-ICl, 128 6 23 mL/min/1.73 m^2) were used as the control group. The results presented that serum Cys-C level showed a greater sensitivity (93.4%) than serum creatinine level (86.8%). In addition, serum Cys-C showed the greatest proportion of increased values in patients with impaired renal function (100%) compared with serum creatinine level (92.15%). Serum Cys-C levels started to increase to greater than normal values when GFR was 88 mL/min/1.73 m2, whereas serum creatinine level began to increase when GFR was 75 mL/min/1.73 m2. The authors concluded that that measurement of serum Cys-C may be useful to estimate GFR, especially to detect mild reductions in GFR, and therefore may be important in the detection of early renal insufficiency in a variety of renal diseases for which early treatment is critical.,

In Florida, Dharnidharka, et al., (2002). performed a meta-analysis of available data from various studies to compare the accuracy of Cys-C and Cr in relation to a reference standard of GFR. A bibliographic search showed 46 articles until December 31, 2001. The data retrieved from eight other studies presented and published in abstract form. The result showed that the overall correlation coefficient for the reciprocal of serum Cys-C (r = 0.816; 95% confidence interval [CI], 0.804 to 0.826) was superior to that of the reciprocal of serum Cr (r = 0.742; 95% CI, 0.726 to 0.758; P < 0.001). Similarly, receiver operating characteristic (ROC)-plot area under the curve (AUC) values for 1/Cys C had greater identity with the reference test for GFR (mean ROC-plot AUC for Cys-C, 0.926; 95% CI, 0.892 to 0.960) than ROC-plot AUC values for 1/Cr (mean ROC-plot AUC for serum Cr, 0.837; 95% CI, 0.796 to 0.878; P < 0.001). Immunonephelometric methods of Cys-C assay produced significantly greater correlations than other assay methods (r = 0.846 versus r =

0.784; P < 0.001). In Conclusion: In this meta-analysis using currently available data, serum Cys-C is clearly superior to serum Cr as a marker of GFR measured by correlation or mean ROC-plot AUC.

In Amsterdam, Hoek, et al., (2003) conducted a study to investigate the usefulness of plasma Cys-C determination in a cross-sectional analysis comparing plasma Cys-C with plasma creatinine, and the Cockcroft and Gault formula for the estimation of glomerular filtration rate. The sample obtained from 93 consecutive patients seen for GFR determination and from 30 patients with diabetes mellitus type 2, of whom 23 were investigated a second time after 2 years. GFR was determined with $[^{125}I]$ iothalamate. Plasma creatinine was determined enzymatically and the creatinine clearance calculated according to C&G. Cys-C was measured with a particle-enhanced immunonephelometric method. The study demonstrated that Cys-C shows a high correlation with GFR. With a very simple formula, Cys-C gives a good estimate of GFR, more accurate and precise than C&G. Because biological variation is low, Cys-C gives also a good assessment of GFR changes during follow-up. Cys-C is the preferred endogenous parameter for GFR.

In Germany, Herget-Rosenthal et al., (2005). conducted a study on 85 patients at high risk to develop ARF to evaluate serum Cys-C as a marker to detect ARF and to test whether Cys-C could detect ARF earlier than serum creatinine. The study showed that the increase of Cys-C significantly preceded that of creatinine. Specifically, serum Cys-C increased already by ≥ 50% 1.5 ± 0.6 days earlier compared to creatinine. Serum Cys-C demonstrated a high diagnostic value to detect ARF as indicated by area under the curve of the ROC analysis of 0.82 and 0.97 on the two days before the R-criteria was fulfilled by creatinine. Cys-C detected ARF according to the R-criteria with a sensitivity of 55% and 82% on these days, respectively. Cys-C also performed excellently, detecting ARF defined by the I- and F-criteria two days prior to creatinine, and moderately well predicting renal replacement therapy in the further course of ARF. In conclusion, Serum Cys-C is a useful detection marker of ARF, and may detect ARF one to two days earlier than creatinine.

In Spain, Villa et al., (2005). to analyze the utility of serum Cys-C as a marker of renal function in these patients.Serum creatinine, serum Cys-C and 24-hour creatinine clearance (CCr) were determined in 50 critically ill patients (age 21–86 years; mean Acute Physiology and Chronic Health Evaluation II score 20 ± 9). They did not have CRF but were at risk for developing renal dysfunction. The result showed that serum creatinine, serum Cys-C and CCr (mean ± standard deviation [range]) were 1.00 ± 0.85 mg/dl (0.40–5.61 mg/dl), 1.19 ± 0.79 mg/l (0.49–4.70 mg/l), and 92.74 ± 52.74 ml/min per 1.73 m2 (8.17–233.21 ml/min per 1.73 m2), respectively. serum Cys-C correlated better with GFR than did creatinine (1/Cys-C versus CCr: r = 0.832, P < 0.001; 1/creatinine versus CCr: r = 0.426, P = 0.002). Cys-C was diagnostically superior to creatinine (area under the curve [AUC] for cystatin C 0.927, 95% confidence interval 86.1–99.4; AUC for creatinine 0.694, 95% confidence interval 54.1–84.6). Half of the patients had acute renal dysfunction. Only five (20%) of these 25 patients had elevated serum creatinine, whereas 76% had elevated serum Cys-C levels (P = 0.032). The study indicated that Cys-C is an accurate marker of subtle changes in GFR, and it may be superior to creatinine when assessing this parameter in clinical practice in critically ill patients.

In Bosnia and Herzegovina, Resic, H., and Mataradzija A. (2006). conducted a study to evaluate the use of Cys-C as a renal marker of the GFR in patients with various degrees of kidney failure. The study included a total of 104 patients (various etiology of kidney disease) with different degrees of kidney failure. All of them were on conservative treatment and 10 healthy patients will comprise the control group. Mean values of Cys-C and creatinine in serum has been measured and compared to endogeneus creatinine clearance. There were 104 patients tested in total with various etiology of kidney disease. The study showed the presence of a significant correlation between creatinine clearance and creatinine r = 0, 663 p < 0, 001, and between creatinine clearance and Cys-C r = 0,765, p < 0, 001 in patients will different degrees of chronic kidney failure (CKF). Correlation between creatinine clearance and Cys-C was significantly better than between serum creatinine p < 0, 05. The author concluded that the level of Cys-C in serum is better marker of kidney function than the level of creatinine in serum. Having in mind that this is faster and cheaper method it could find wider application in everyday clinical

practice, especially in elderly (or in children) where it is often impossible to accurately collect 24-hour urine (incontinence).

In Solvenia, Hojs et al., (2006). conducted a study to compare serum creatinine and serum Cys-C with ^{51}CrEDTA clearance in a population of patients with mild (stage 2 of CKD) to moderate (stage 3 of CKD) impairment kidney function. and to compare serum Cys-C and creatinine clearance calculated from the C&G and MDRD formulas with ^{51}CrEDTA clearance in the same patients. A total of 164 patients with CKD stages 2–3 (GFR 30–89 ml/min/1.73m2), who had performed ^{51}Cr-labelled ethylenediaminetetra-acetic acid clearance, were enrolled. In each patient, serum creatinine and Cys-C were determined. Creatinine clearance was calculated using the Cockcroft–Gault (C&G) and the modification of diet in renal disease (MDRD) formulas. The study indicated that the receiver operating characteristic (ROC) curve analysis (cut-off for GFR 60 ml/min/1.73m^2) showed that serum Cys-C had a significantly higher diagnostic accuracy than serum creatinine (P¼0.04) and calculated creatinine clearance from the C&G formula (P<0.0001), though only in female patients. No difference in diagnostic accuracy was found between serum Cys-C and creatinine clearance calculated from the MDRD formula. The authors concluded that serum Cys-C is a reliable marker of GFR in patients with mildly to moderately impaired kidney function and has a higher diagnostic accuracy than serum creatinine and calculated creatinine clearance from the C&G formula in female patients.

In Boston, Stevens, et al., (2008). preformed a Pooled Analysis study of 3418 Individuals with CKD Participants screened for three CKD studies in the US (n=2980) and a clinical population in Paris, France (n=438), to Estimate GFR using Serum Cys-C Alone and in Combination with Serum Creatinine. GFR Estimated using the four new equations based on Serum Cys-C alone, Serum Cys-C, Serum Creatinine or both with age, sex and race. GFR was measured using using urinary clearance of 125I-iothalamate in the US studies and chromium-ethylenediaminetetraacetate (51Cr-EDTA) in the Paris study, while Serum Cys-C was measured by Dade Behring assay. standardized Serum Creatinine. The study showed that Cys-C alone provides GFR estimates that are nearly as accurate as

Serum Creatinine adjusted for age, sex and race thus providing an alternative GFR estimate that is not linked to muscle mass.

In India, Kumaresan et al., (2011). carried out a study is aimed to compare the diagnostic performance of serum Cys-C and creatinine with measured GFR and estimated GFR in subjects of Indian origin. One hundred and six CKD patients (82 males, 24 females) were enrolled and categorized into three groups based on age. The eGFR was calculated using Cockcroft-Gault (CG) and MDRD formulae. Serum cys-C was measured with a particle-enhanced nephelometric immunoassay (PENIA) method. GFR was measured using 99 mTC – diethylene triamine penta aceticacid (DTPA) renal scan method. The study result demonstrated that Serum Cys-C showed significant correlation with measured GFR in all the three groups (r=-0.9735, r=-0.8975 and r=-0.7994, respectively) than serum creatinine (r=-0.7380, r=- 0.6852 and r=-0.5127, respectively). In conclusion, Serum Cys-C showed a high correlation with measured GFR in young and older patients with CKD than creatinine. Thus, Cys-C is a good alternative marker to creatinine in CKD patients.

In India, Kumaresan et al., (2012). conducted a study to compare the diagnostic performance of Cys-C based prediction equations and creatinine based prediction equations with isotope GFR in 182 CKD patients. GFR patients. GFR was estimated using two equations (LeBricon and Hoek) that are based on serum Cys-C and two equations (Cockcroft-Gault and MDRD) that are based on serum creatinine in measured by using radiolabelled diethylenetriaminepentaaceticacid (99 mTc-DTPA) renal scan method is used as the standard for comparison. The study showed that the average isotope GFR was 33.81 (ranged from 6 - 110 ml/min/1.73m2). The Cys-C based equations correlated well with all stages of the CKD than creatinine based equations. The authors concluded that Cys-C based formulae provides a better diagnostic performance than creatinine based equations for GFR calculation in CKD population. The LeBricon formula is most accurate.

In China, Zhang, et al., (2013). performed a meta-analysis study to evaluate the diagnostic value of serum Cys-C and serum creatinine for estimating GFR in patients with CKD. The data were searched from Google Scholar, PubMed, Cochrane Library and China National Knowledge Infrastructure databases, to

identify randomized controlled trials that determined the diagnostic value of Cys-C and Creatinine, for estimating GFR in patients with CKD. The inclusion criteria were met by 17 studies (total number of patients with CKD, 2521). The meta-analysis showed that when the diagnostic cut-off value of GFR was 80 - 90 ml/min/1.73m^2, the heterogeneity was modest for Cys-C (I^2= 48%, summary sensitivity [SEN]= 0.803, summary specificity [SPE]= 0.821), but there was no heterogeneity for serum creatinine (I^2= 0.0%, SEN= 0.697, SPE= 0.787). Meta-analysis of the studies demonstrated a significant difference between patients with CKD and controls, for Cys-C and serum creatinine. In Conclusions, the meta-analysis demonstrated significant correlations between Cys-C, serum creatinine and GFR. Cys-C was more sensitive, but less specific, than serum creatinine for the estimation of GFR.

In Iran, Khorgami, et al., (2013). performed a cross-sectional study, study to determine whether Cys-C based equation could be used as an indicator for renal function in hemodialysis patients compared to MDRD equation; and whether Cys-C, a dialyzable molecule, was related to Kt/V, the marker for dialysis adequacy. The study population were 98 patients on chronic HD. Plasma levels of urea and creatinine were measured before and after dialysis, and Cys-C was measured before dialysis. GFR was calculated and compared. The study showed that GFR was estimated at 6.05 ± 2.36 and 5.83 ± 2.19 cc/min by MDRD and Cys-C based formulas, respectively, with a significant correlation (r = 0.51; P < 0.001). Serum Cys-C level was 9.74 ± 2.47 mg/L which showed significant reverse correlation with both MDRD (r = -0.46; P < 0.001) and Cys-C based formulas (r = -0.87; P < 0.001). Neither creatinine nor serum Cys-C showed correlation with Kt/V, as the marker of dialysis adequacy. The authors concluded that Serum Cys-C may be considered as an indicator of renal function in patients under maintenance HD.

In Baghdad, AL-Hussaini, (2013). carried out a study evaluate serum Cys-C and serum creatinine in pre and post HD patients, as well as to investigate a possible correlation between serum Cys-C and creatinine. A total thirty-five patients with twenty normal subjects were included in this study. All patients diagnosed with CKD (pre and post- HD). ELISA (enzyme linked immune sorbent assay) technique used for measurement of serum Cys-C. Serum creatinine was determined by using

colorimetric method. The results showed that the level of serum Cys-C were significantly higher (p < 0.001) in the post-HD patients as compared with the pre-HD patients and healthy subjects measured of Cys-C in pre and post HD. The author concluded that Serum Cys-C significantly predicted renal dysfunction. Cys-C considered more sensitive and detectable than creatinine for kidney dysfunction, and measurement of serum Cys-C in HD patients might help to the overall clinical status of the patients.

In India, Hari, et al., (2014). performed a study to on 100 CKD patients to compare performance of combined creatinine and Cys-C -based equation with equations based on either Cys-C or creatinine alone, in early CKD. The study showed that Cys-C based equation had less bias (1.9 vs. 12.4 ml/min/1.73 m^2), and higher precision (13.1 vs. 25.6 mL/min/1.73 m^2) and accuracy (92.1% vs. 75.7%) than creatinine-based equation. The combined Cys-C and creatinine equation had bias (1.4 mL/ min/1.73 m^2) precision (15.2 mL/min/1.73 m^2) and accuracy (91.2%) similar to Cys-C-based equation. The authors concluded that Cys-C -based equation has a better performance in estimating GFR than creatinine-based equation in children with early CKD. Addition of creatinine equation does not improve the performance of the Cys-C -based equation.

In UK, Vilar et al., (2014). performed a study to determine Cys-C kinetics in HD to understand whether blood concentrations may predict residual renal function and middle-molecule clearance. Cys-C removal and rebound kinetics were studied in 24 patients on high-flux HD or hemodiafiltration to determine whether Cys-C concentrations are predictable, an iterative two-pool mathematical model was applied. The study showed that Cys-C was cleared effectively, although less than β_2-microglobulin (reduction ratios6SD are 39% ± 11 and 51% ± 11). Cys-C rebounded to 95% ± 5% of predialysis concentration by 12 hours postdialysis. The two-pool kinetic model showed excellent goodness of fit. Modeled extracellular Cys-C pool volume is smaller than that predicted, comprising 25.5% ± 9.2 of total body water. Iterated parameters, including non-renal clearance, showed wide inter-individual variation. Modeled non-renal clearance was substantially higher than renal clearance in this population at 25.1 ± 6.6 ml/min per 1.73 m^2 body surface area. In

Conclusions, Plasma Cys-C levels may be used to measure middle-molecule clearance. Levels rebound substantially post-dialysis and plateau in the interdialytic period. At low GFR, nonrenal clearance predominates over renal clearance, and its interindividual variationwill limit use of Cys-C to predict residual renal function in advanced kidney disease.

In India, Maheshwari, et al., (2014). conducted a study to determine per dialysis Cys C reduction ratio (RR) in low flux group and to compare it with urea, marker of dialysis adequacy in 37 ESRD patients on hemodialysis. They found that the URR is72.273±14.686% in low flux group & the Cys CRR is -9.7 ± 6.7 %. The paradoxical increase in Cystatin C in the low flux group shows the ineffective clearance of middle molecules by low flux dialysers which is associated with dialysis related morbidity & mortality. Hence, Cys CRR could be applied as a surrogate marker for the inadequacy of dialysis.

In Haryana, Dhupper, et al., (2015). performed a study on 40 known patients of CKD attending nephrology unit of medicine at PGIMS, Rohtak were enrolled as cases for this hospital based cross sectional study, and 40 age and sex matched healthy subjects were taken as controls. Both the cases and controls analyzed for serum creatinine, Cys-C and urine creatinine. Estimated GFR (eGFR) calculated from MDRD equation along with creatinine clearance using standard formula. The study showed that Serum Cys-C increased with stage wise progression of CKD, Cys-C has also shown more significant Pearson correlation with eGFR than serum creatinine. The study concluded that Cys-C has small variability and is unaffected by preanalytic factors such as routine clinical storage conditions, freezing and thawing cycles, or interfering substances, such as bilirubin or triglycerides. Thus, it may be better to use Cys-C for staging of CKD than indirect measurement of eGFR with serum creatinine based equations. Haryana.

In india, Maheshwari et al., (2016). carried out a study to determine Cys-C reduction ratio (CCRR) in both LF and HF dialyzers groups and to compare it with other markers of dialysis adequacy. 73 patients were subjected to both LF and HF hemodialysis 2 weeks apart. Serum urea, creatinine and Cys-C were measured pre

and post-dialysis. The result showed that urea and CrRR were 72.3 ± 14.7% and 62.5 ± 13%, respectively in the LF group. The CCRR was − 9.7 ± 6.7% and 29.2 ± 11% in LF and HF hemodialysis, respectively. The statistically significant decrease in CCRR in the HF group shows the effective clearance of middle molecules (MM) by HF dialyzers. Hence, CCRR could be applied to measure the MM clearance in HF hemodialysis. In conclusion, this study highlights the significance of Cys-C as an important dialysis adequacy marker replacing the conventional markers such as urea and creatinine in HF hemodialysis. Among the middle molecules Cys-C scores over beta-2 microglobulin.

In Iran, Amini, et al., (2016). carried out a cross-sectional multicenter national study aimed to evaluate the hemodialysis adequacy in 4004 ESRD patients 2345 men (58.6%) and 1659 women (41.4%) in Iran. The results reported that Bicarbonate-based solutions and low-flux membranes were prescribed for 77.0% and 97.6% of the patients, respectively. The mean blood flow rate was 242.9 ± 39.2 mL/min. The mean length of hemodialysis session was 229.2 ± 22.2 minutes. The mean urea reduction ratio and Kt/V were calculated to be 61.0 ± 11.8% and 1.2 ± 0.4, respectively. A Kt/V less than 1.2 and a urea reduction ratio less than 65% were found in 56.7%, and 65.2% of the hemodialysis patients, respectively.

Chapter -3
Subjects and Methods

Chapter -3

Subjects and methods

3.1 Study design

The present study was observational cross section (pre and post design).

3.2 Study population

The study population was comprised chronic kidney disease with end-stage renal disease patients on regular twice or three-weekly 4-hour per sessions hemodialysis for at least 3 months, from both genders, aged more than 12 years attending kidney dialysis unit at Al-Shifa hospital Gaza governorate.

3.3 Sample size

A total of 80 chronic kidney disease with end-stage renal disease patients, divided to 40 male group and 40 female group, whose aged more than 12 years. All patients recruited from kidney dialysis unit at Al-Shifa hospital Gaza governorate. the marker dialysis adequacy measurement in a single HD treatment at pre-dialysis and post-dialysis on consecutive.

3.4 Inclusion criteria

− CKD with ESRD patients whose aged more than 12 years old, attending kidney dialysis unit at Al-Shifa hospital Gaza governorate.

− Patients on regular twice or three-weekly 4-hour per sessions HD for at least three months.

− Patients with other CKD such as diabetes, and hypertension, and anemia.

3.5 Exclusion criteria

− CKD Patients whose aged below12 years old.

− CKD Patients whose aged above 12 years old and not need dialysis.

− Patients who take hormone replacement therapy or corticosteroid therapy.

− Patient with liver cirrhosis, heart disease, thyroid dysfunction, hematologic disorder or malignant disease and pregnant women was excluded from the study

to eliminate potential confounding factors which may influence heart function and plasma biomarkers.

3.6 Ethical consideration

The necessary approval to conduct the study was obtained from Helsinki committee in the Gaza Strip optional (**Appendix: 2**). Coordination with the ministry of health (MOH) was fulfilled (**Appendix: 3**). Coordination with the laboratory of El-wafa medical rehabilitation and specialized surgery hospital was fulfilled (**Appendix: 4**). Parents of the participants was given a full explanation about the purpose of the study, assurance about the confidentiality of the information obtained through the questionnaire and blood analysis, and that they have the right to refuse to participate or to drop out in any phase of the study.

3.7 Data collection

3.7.1 Questionnaire interview

A meeting interview was used for filling a questionnaire which designated for matching the study need. The questionnaire **(Appendix: 1)** was based on chronic kidney disease flow sheet with some modifications. All interviews were conducted face to face by the researcher himself through using a clear Arabic language. During the study the interviewer was explained to the Parents of the participants any of the confused questions that was not clear to them. Most questions were the yes/no question which offers a dichotomous choices and multiple choice **(Backestrom and Hursh-Cesar, 2012)**. The validity of the questionnaire was tested by six specialists in the fields of nephrology, epidemiology, public health, biochemistry and nutrition. The questionnaire included questions on sociodemographic data like (name, Age, sex, weight, height, education, employment, residence family income/month and family history of CKD), and clinical data (time at diagnosis and duration of CKD, duration and history of dialysis, session per week, membranes types). In addition, it includes other information like type of family history, medication and complication of CKD (retinopathy, neuropathy, CVD, bone disease and obesity). Most questions were yes or no question that offers a dichotomous choice.

3.7.2 Measurement of blood pressure

Blood pressure (systolic blood pressure and diastolic blood pressure) were obtained twice measurement in a single HD treatment (pre-dialysis and post-dialysis), from the individuals in the sitting position by using of dialysis machine monitor blood pressures screen.

3.7.3 Body mass index

Body mass index (BMI) was calculated as the ratio of body weight in Kg/height in meter square (BMI = weight (kg) /(height in meter)^2). The subjects were asked to remove shoes and heavy clothes before measurement of weight and height. This is the world health organization's (WHO) recommended body weight based on BMI values for adults. people with BMI, less than 15.0 kg/m² (Very severely underweight), Between 15.0-16.0 kg/m² (severely underweight), Between 16.0-18.5 kg/m² (underweight), Between 18.5-25.0 kg/m² (normal healthy weight), Between 25.0-30.0 kg/m² (overweight), Between 30.0-35.0 kg/m² (moderately obese), Between 35.0 – 40.0 kg/m² (severely obese), Over 40.0 kg/m² (very severely obese) **(World Health Organization, 2017).**

3.7.4 Specimen collection and biochemical analysis

Convenient sampling method was used for selection of the study population, in order that every individual has to meet the criteria of being included in the sample subjects.

3.7.4.1 Blood samples

Blood samples were collected from male and female CKD with ESRD patient's fasting and non-fasting attending Kidney dialysis unit. about 10 milliliters arteriovenous fistula (AVF) blood samples were collected from each subject, before and after dialysis into one EDTA tube vacutainer and other one Plain tube vacutainer. the blood sample was drawn by the researcher himself under quality control and safety procedure. The blood in Plain tube will be left for a while without anticoagulant to allow blood to clot. Then serum samples was obtained by centrifugation at room temperature at 4000 rpm/10 minutes and collected into two cryo-plastic tubes, then Serum samples were stored at -70°C for no more than one

month until the time of performing the analysis were done in the laboratory at Al-Shifa hospital Gaza governorate ,for biochemical analysis before and after, serum creatinine, urea, BUN, uric acid, total protein, albumin, phosphorus, tCa^+, iCa^{++}, Na^+, K^+, Cl^-, blood sugar, cholesterol, triglycerides, high-density lipoprotein (HDL-C), low density lipoprotein (LDL-C) and Cys-C were analyzed. The blood in EDTA tube was used to perform pre-dialysis complete blood count (CBC) was done in the same day of collection. All of hematological analysis and biochemical analyses were done in the laboratory of El-wafa Medical Rehabilitation &specialized surgery hospital in Gaza.

3.8 Calculated measurements

- Blood Urea Nitrogen **(BUN)** was calculated by (The urea value multiply by factors (0.467)) or divided by value (2.14).
- Low density lipoprotein **(LDL-C)** was calculated by Friedewald equation:
  LDL (mg/dl) = Cholesterol – (HDL + triglycerides /5).
- Body mass index (BMI) was calculated by
  BMI = (weight in kilograms / square of the height in meters).
- Calculation of chemical tests for serum urea, creatinine, uric acid, cholesterol triglycerides, total protein, albumin, phosphorus, blood sugar and high-density lipoprotein (HDL-C) were performed by the auto chemistry analyzer according to beer's law after calibration and adjustment by using a specific program of every test inserted to the instrument.
- **Beer's law**: The concentration of colorimetric test =
$$\frac{\text{(Abs. of sample) X (Concentration of Calibrator)}}{\text{Abs. of Calibrator}}$$
- The efficacy of dialysis formulae of interest for dialysis adequacy was then assessed by calculating the reduction ratio for serum urea (URR), creatinine (CrRR) and Cys-C (CCRR) as shown below the same formula:
- **Urea reduction ratio:** was calculated by

$$URR\% = 100 \, X \left(1 - \frac{post \, D. \, urea}{Pre \, D. \, urea} \right) \quad \text{Or} \quad URR\% = 100 \, X \left(\frac{(Pre \, D. \, urea) - (Post \, D. \, urea)}{Pre \, D. \, urea} \right)$$

$$URR\% = 100 \, X \left(1 - \frac{post \, D. \, BUN}{Pre \, D. \, BUN} \right) \quad \text{Or} \quad URR\% = 100 \, X \left(\frac{(Pre \, D. \, BUN) - (Post \, D. \, BUN)}{Pre \, D. \, BUN} \right)$$

- **Creatinine reduction ratio:** was calculated by

$$CrRR\% = 100\ X\left(1 - \frac{post\ D.\ \text{Creat.}}{\text{Pre D. Creat.}}\right) \text{ Or } CrRR\% = 100\ X\left(\frac{(Pre\ D.\text{Creat.}) - (Post\ D.\text{Creat.})}{Pre\ D.\text{Creat.}}\right)$$

- **Cystatin C reduction ratio:** was calculated by

$$CCRR\% = 100\ X\left(1 - \frac{post\ D.\text{Cys}-\text{C}}{\text{Pre D.Cys}-\text{C}}\right) \text{ Or } CCRR\% = 100\ X\left(\frac{(Pre\ D.\text{Cys}-\text{C}) - (Post\ D.\text{Cys}-\text{C})}{Pre\ D.\ \text{Cys}-\text{C}}\right)$$

- **Kt/V Index:** was calculated by

$$Kt/V = ln\left(\frac{C_0}{C_t}\right) \qquad \text{Or} \qquad Kt/V = -Ln\ (1 - URR)$$

Where **C₀** and **Cₜ** represent the pre-dialysis and post-dialysis serum urea.

- **Single-pool Kt/V (Sp-Kt/v):** The equation below is an example of a simplified, second generation logarithmic urea kinetic modeling (UKM) formula used to calculate spKt/V was used the natural logarithm formula of Daugirdas II,

$$SpKt/V = -Ln\ (R - 0.008 \times t) + (4 - 3.5 \times R) \times \frac{UF}{W}$$

Where **(ln)** is the natural logarithm, **(R)** is (post-dialysis Urea / pre-dialysis Urea), **(t)**is session length time in hours, **(UF)**is the is ultrafiltration volume (liters), **(W)** is post-dialysis weight in kilograms.

3.9 Materials and instruments

3.9.1 Chemicals and reagents

Chemicals and reagents used in this study are shown in **(Table: 3.1)**

Table (3.1): Chemicals and reagents used in the present study

Reagent	Supplier
CBC	Diatron Solution 3diff, Abacus Junior30, Hungary
ichromx™ Cystatin C	Boditech, Med Inc, Korea
Blood Glucose	AMS -Enzymatic colorimetric method, Italy
Urea-UV	AMS -Enzymatic method, Italy
Creatinine	AMS- Kinetic colorimetric method, Italy
Uric Acid	AMS -Enzymatic colorimetric method, Italy
Cholesterol-total	AMS -Enzymatic colorimetric method, Italy
Triglycerides	AMS -Enzymatic colorimetric method, Italy
High-Density Lipoprotein (HDL-C),	Diasys, Liquid HDL precipitant method Halzheim, Germany
Total Protein	AMS - Colorimetric method, Italy

Reagent	Supplier
Albumin	AMS -Colorimetric method, Italy
Phosphorus-UV	AMS -Enzymatic colorimetric method, Italy
Sodium, Potassium Calcium, Ionize Calcium, Chloride	Meizhou coenley Hi Tech Co.,China
Quality control	
Cornley electrolytes controls (3 level)	Meizhou coenley Hi Tech Co.,China,
RD, systems CBC controls (3 level)	R and D systems, USA
Normal chemistry control	Labkit, Labtrol normal, chemelex,S.A,Spain
Pathological chemistry control	Labkit, Labtrol Pathological, chemelex,S.A,Spain
Cystatin C control (2 level)	Diazyme laboratories, Shanghai-china

3.9.2 Instruments and equipment

The main Instruments and equipment that were used are listed in **(Table: 3.2)**

Table (3.2): Main equipment used in the present study

Equipment and Instrument	Manufacturer
Hematology Analyzer (CBC)	Abacus Junior30 ,Diatron 3diff, Hungary
Auto chemistry analyzer	Rayto-chemray 240,Hamburg Germany
Electrolyte analyzer	Cornley-AFT-500 Electrolyte,(Nanshan industrial),China
Ichroma TM reader class(I)	I chromx ^{TM}, reader, Korea
Centrifuge TD4N	TD4N (16 tubes), hangsh ,China
Refrigerator with 2-8 Cº	Haier,Bio-Medical,China Lavalux, Refrigerator, Turkey
Deep Freezer -80 Cº	Haier,Bio-Medical, ultra-low temp. freezer, China .
Vortex Mixer	Vortex Mixer, China
Shaker	Shaker, China
Micropipettes different size	Stan-Bio laboratory,USA

3.10 Hematological analyses

3.10.1 Complete blood count (CBC)

Blood CBC samples were measured using automatic hematology analyzer for measurement hemoglobin concentration and other whole blood component concentrations (Abacus Junior30, Diatron 3diff, Hungary). three levels of hematological controls (R&D systems, USA), high, normal, and low were used in each run of CBC.

3.11 Biochemical analysis

Serum glucose, urea, creatinine, uric acid, cholesterol, triglycerides, HDL-C, total protein, albumin and phosphorus were analyzed using Auto chemistry analyzer (Rayto-chemray 240, Hamburg Germany). The blood urea nitrogen was calculated by using equation (The urea value multiply by factors (0.467) [BUN =Urea x 0.467]. and then LDL-C was calculated. The concentration of LDL-C was calculated from the results of a profile including total cholesterol, HDL and triglycerides using the Friedewald equation (LDL = cholesterol – (HDL + triglycerides/5) **(Kaplan and Szabo, 1983).** Two levels of lyophilized multi-control sera (Labtrol, chemelex, S. A, Spain). normal and pathological levels were analyzed with each run.

The concentration of Serum glucose, urea, creatinine, uric acid, cholesterol, triglycerides, HDL-C, total protein, albumin and phosphorus which performed by the automated analyzer calculated according to beer's law after calibration and adjustment of the photometers against water blank using a specific program of every test inserted to the instrument. Serum Cys-C was analyzed using a fluorescence immunoassay (FIA) analyzer (ichromx^{TM} reader, class$^{(I)}$, Korea). two levels of human-based control serum were analyzed with each run. Electrolytes parameters (sodium, potassium, total calcium, ionize calcium, chloride) were analyzed using ion selective electrode by electrolyte analyzer (Cornley-AFT-500 Electrolyte, (Nanshan industrial), China). three levels of controls (I, II, III) were analyzed with each run.

3.11.1 Determination of serum cystatin C

Serum Cys-C was determined quantitative by a fluorescence Immunoassay (FIA) **(Laterza et al., 2002).** using a commercially available kits (ichroma^{TM}, Boditech, Med Inc, Korea).

Principle

Serum Cys-C was determined in sample by used a sandwich immune-detection method. in buffer the detector recombinant protein binds to antibody in sample, forming recombinant protein-antibody complexes, and migrates onto nitrocellulose matrix to be captured by the other immobilized-antigen on test strip. The more antibody in sample forms the more recombinant protein-antibody complexes and leads to stronger intensity of fluorescence signal on detector recombinant protein,

Reagents and components

ichroma™ Cystatin C	
Cartridges(test strip)	the membrane which was anti human cystatin C at the test line, while chicken IgY at the control line.
Detection Buffer Tubes	Anti-human cystatin C-fluorescence conjugate. Anti-chicken IgY-fluorescence conjugate. Bovine serum albumin (BSA) as a stabilizer. Sodium azide in phosphate buffered saline (PBS) as a preservative
ID chip	Insert into the ID chip port of the instrument for ichroma™ reader to Cystatin C test .

Assay procedure

o All samples, cartridge and the detection buffer tubes were allowed to reach at room temperature for at least 30 minutes.

o Transferred 10 µL of samples (serum/controls) were used micropipettes to the detection buffer tubes.

o Each well of detection buffer tubes were closed and shaking it about 10 times. all of the sample mixture must be used immediately.

o Transferred out 75 µL of the samples mixture were added gently into each sample well in the Test Cartridge.

o The sample cartridge was incubated at room temperature for 10 minutes.

o ID chip was inserted into ID chip port of the instrument for ichroma™ cys-C test.

o The sample cartridge was read by insert it into the cartridge holder of the instrument in the direction of the arrow marked on cartridge.

o Instrument for ichroma™ tests were started scan the sample cartridge immediately.

o Instrument for ichroma™ tests were read the test result on the display screen.

Calculation

Cys-C concentration terms of mg/L results were calculated automatically on the displays instrument for ichroma™

Reference value

Concentration of cystatin C in healthy individuals
0.56 – 0.90 mg/L

3.11.2 Determination of serum glucose

Serum glucose was determined by enzymatic colorimetric method (glucose oxidase (GOD)) **(Trinder, 1969).** using a commercially available kits (AMS, Italy).

Principle

Glucose was transformed by catalytic action of glucose oxidized (GOD) to gluconic acid and hydrogen peroxide (H_2O_2), which in the presence of peroxidase (POD) it is reacts with phenol and 4-aminoantipyrine to form a red complex (quinoneimine).

$$Glucose + O_2 \xrightarrow{\text{Glucose oxidase}} Gluconic\ acid + H_2O_2.$$

$$2H_2O_2 + Phenol + 4\text{-Aminoantipyrine} \xrightarrow{\text{Peroxidase}} Quinoneimine + 4H_2O.$$

The intensity of the color is proportional to glucose concentration in the sample.

Reagents and components

Reagents	Component	Concentration
Reagent-A:	Phosphate buffer	25 g/l
	Phenol	< 0.9 g/l
	4-Aminoantipyrine	0.4 mmol/l
	Glucose oxidase	≥ 30 kU/l
	Peroxidase	≥ 1 kU/l
	pH	7.4
	NaN_3	0.95 g/l
Standard (Std.):	D-Glucose	100 mg/dl (5.55 mmol/l)
	Benzoic acid	< 14.7 mmol/l

Reagent preparation:

Glucose reagent was liquid ready to use.

Assay procedure

About 0.5 ml of serum was transferred to the chemistry (Autoanalyzer Rayto-chemray240, Hamburg, Germany). to perform the test according to these parameters:

Parameter	Value
Reaction type	End point
Wavelength (nm)	510 nm
Temperature	37 C$^\circ$
Direction	Increase
mix and Incubation time (minutes)	10 min

Parameter	Value
Unit	(mg/dl)
Reagent volume (µl)	300 µl
Sample volume (µl)	3 µl
Calibrator type	Linear
absorbance (A) was read of the standard and samples at against blank	

Calculation

$$\text{Serum Glucose, (mg /dl)} = \frac{\text{Abs. sample}}{\text{Abs. standard}} \times 100$$

Reference value

Glucose (serum/plasma)	
Adults:	FBS: 70 – 115 mg/dl PPBS: >140 mg/dl

3.11.3 Determination of serum urea

Serum urea was determined by enzymatic U.V. method (urease/glutamate dehydrogenase (GLDH)) **(Kaplan, 1984).** using a commercially available kits (AMS, Italy).

Principle

Serum urea was cleaved enzymatically into NH_4^+ and CO2. NH_4^+ reacted with α- ketoglutarate in a reaction catalyzed by Glutamate dehydrogenase (GLDH) with simultaneous NADH to NAD^+

$$\text{Urea + 2 H}_2\text{O} \xrightarrow{\textbf{Urease}} 2\ NH_4^+ + 2\ HCO3^-$$

$$\text{2-Ketoglutarate} + NH_4^+ + \text{NADH} \xrightarrow{\textbf{GLDH}} \text{L-Glutammate} + NAD^+ + H_2O$$

The decrease in NADH absorbance is proportional to urea level in the sample.

Reagents and components

Reagents	Component	Concentration
Reagent-A:	TRIS	150 mmol/l
	2-Ketoglutarate	8.75 mmol/l
	ADP	0.75 mmol/l
	Urease	≥ 7.5 kU/l
	GLDH (Glutamate-dehydrogenase)	≥ 1.25 kU/l
	Sodium azide	≤ 0.95 g/l
	pH	7.8

Reagents	Component	Concentration
Reagent-B:	NADH	1.32 mmol/l
	Sodium hydroxide	$\geq 0.1\%$
Standard (Std.):	Urea	50 mg/dl

Preparation and stability of working reagent

The working solution was prepared by mixing 4 parts of Reagent-A with 1 part of Reagent-B.   Stability: 5 days at 15-25 C °,

4 weeks at 2-8 C °

Assay procedure

About 0.5 ml of serum was transferred to the chemistry (Autoanalyzer Rayto-chemray240, Hamburg, Germany). to perform the test according to these parameters:

Parameter	Value
Reaction type	fixed time
Wavelength (nm)	340 nm
Temperature	37 C°
Direction	decrease
mix and Incubation time (seconds)	30 sec Abs.(A_1) ,after 60sec Abs.(A_2)
Unit	mg/dl
Reagent volume (μl)	300 μl
Sample volume (μl)	3 μl
Calibrator type	Linear
absorbance (A) was read of the standard and samples at against blank	
Determine: A = [(A_1–A_2) sample or standard]-[(A_1–A_2) blank]	

Calculation

$$\text{Serum Urea, (mg/dl)} = \frac{\triangle\text{A Abs. sample}}{\triangle\text{A Abs. standard}} \text{ X } 50$$

Conversion factor

Blood Urea Nitrogen (BUN) = Urea [mg/dl] X 0.467

Reference value

Urea & BUN (Serum/Plasma)		
Adults:	Urea :	18 – 53 mg/dl
	BUN:	7 – 21 mg/dl

3.11.4 Determination of serum creatinine

Serum Creatinine was determined by using kinetic test methods without deproteinization according to the Jaffé method **(Fabiny et al., 1971).** using a commercially available kits (AMS, Italy).

Principle

Picric acid in alkaline solution was reacted with creatinine to compose yellow-orange color complex with the alkaline picrate. The intensity of color formed during the fixed time is directly proportional to the amount of creatinine present in the sample.

Creatinine + Picric acid $\longrightarrow$ creatinine picrate complex (yellow-orange colored)

Reagents and components

Reagents	Component	Concentration
Reagent-A:	Sodium hydroxide	1.25 mmol/l
Reagent-B:	Picric acid	20.5 mmol/l
Standard (Std.):	Creatinine	2.0 mg/dl

Preparation and stability of working reagent

The working solution was prepared by mixing equal volumes of 1-part Reagent-A with 1-part Reagent-B, the working reagent is stable for 7 days at 2-8C°.

Assay procedure

About 0.5 ml of serum was transferred to the Chemistry (Autoanalyzer Rayto-chemray240, Hamburg, Germany). to perform the test according to these parameters:

Parameter	Value
Reaction type	fixed time
Wavelength (nm)	510 nm
Temperature	37 C°
Direction	Increase
mix and Incubation time (seconds)	10 sec Abs.(A_1) ,after 60sec Abs.(A_2)
Unit	mg/dl
Reagent volume (µl)	300 µl
Sample volume (µl)	30 µl
Calibrator type	Linear
absorbance (A) was Read of the standard and samples at against distilled water.	
Determine: $\Delta A = [(A_2 - A_1)$ sample or standard]	

Calculation

$$\text{Serum creatinine, (mg/dl)} = \frac{\triangle A \text{ Abs. sample}}{\triangle A \text{ Abs. standard}} \text{ X 2}$$

Reference value

Creatinine (Serum/Plasma)		
Adults:	M:	0.6 – 1.3 mg/dl
	F:	0.5– 1.2 mg/dl

3.11.5 Determination of serum uric acid

Serum uric acid was determined by using enzymatic colorimetric endpoint method with 4-aminofenazone and N-Ethyl-N-(hydroxi-3-sulphopropil)-p-toluidine (ESPT) **(Barham et al., 1972)**. using a commercially available kits (AMS, Italy).

Principle

Uric acid was oxidized by uricase into allantoin with production of hydrogen peroxide, in the presence influence of peroxidase reacts with 4-aminofenazone and N-Ethyl-N-(hydroxi-3 sulphopropil)-p-toluidine (ESPT) to form a blue-violet color:

$$\text{Uric acid} + H_2O + O_2 \xrightarrow{\textbf{Uricase}} \text{Allantoine} + CO_2 + H_2O_2$$

$$\text{ESPT} + \text{4-Aminophenazone} + 2\,H_2O_2 \xrightarrow{\textbf{POD}} \text{Indamine} + 3\,H_2O$$

The intensity of the color measured at 510 nm is proportional to the uric acid concentration in the sample.

Reagents and components

Reagents	Component	Concentration
Reagent-A:	Borate Buffer	180 mmol/l
	Uricase	> 50 U/l
	Cholesterol esterase (CHE)	> 300 U/l
	4-aminophenazonel	0.25 mmol/
	ESPT	1 mmol/l
	Peroxidase (POD)	> 100 U/l
	NaN3	< 0.095 g/l
	pH	7.0
Standard (Std.):	Uric acid	6 mg/dl

Reagent Preparation

Uric acid single reagent was ready to use.

Assay procedure

About 0.5 ml of serum was transferred to the chemistry (Autoanalyzer Rayto-chemray 240, Hamburg, Germany). to perform the test according to these parameters:

Parameter	Value
Reaction type	End point
Wavelength (nm)	510 nm
Temperature	37 C^o
Direction	Increase
Mix and incubation time (minutes)	10 min
Unit	mg/dl
Reagent volume (μl)	400 μl
Sample volume (μl)	8 μl
Calibrator type	Linear
absorbance (A) was read of the standard and samples at against blank	

Calculation

$$\text{Serum Uric acid, (mg/dl)} = \frac{\text{Abs. sample}}{\text{Abs. standard}} \times 6$$

Reference value

Uric acid (Serum/Plasma)		
Adults:	M:	3.6 - 8.2 mg/dl
	F:	2.3 - 6.1 mg/dl

3.11.6 Determination lipid profile measurements

3.11.6.1 Determination of serum cholesterol

Serum total cholesterol was determined by using enzymatic colorimetric method with (cholesterol oxidase (CHOD)) (**Jakobs et al., 1990**). using a commercially available kits (AMS, Italy).

Principle

The measurement was based on the following enzymatic reactions:

Cholesterol esters + H_2O $\xrightarrow{\textbf{CHE}}$ Cholesterol + Fatty acids

Cholesterol + O_2 $\xrightarrow{\textbf{CHOD}}$ Cholest-4-en-3-one + H_2O_2

$2H_2O_2$ + hydroxybenzoate + 4-AmminoantiPyrine $\xrightarrow{\textbf{POD}}$ Red complex + 4 H_2O

The intensity of the red complex is proportional to the total cholesterol in the sample.

Reagents and components

Reagents	Component	Concentration
Reagent-A:	Good buffer	50 mmol/l
	Cholesterol oxidase (CHOD)	≥ 100 U/l
	Cholesterol esterase (CHE)	≥ 300 U/l
	Hydroxybenzoic acid	12 mmol/l
	4-Amminoantipirine	0.3 mmol/l
	Peroxidase (POD)	≥ 500 U/l
	Sodio azide	≤ 0.095 g/l
	pH	6.7
Standard (Std.):	Cholesterol	200 mg/dl

Reagent preparation

Total Cholesterol single reagent was ready to use.

Assay procedure

About 0.5 ml of serum was transferred to the Chemistry (Autoanalyzer Rayto-chemray 240, Hamburg, Germany). to perform the test according to these parameters:

Parameter	Value
Reaction type	End point
Wavelength (nm)	510 nm
Temperature	37 C°
Direction	Increase
mix and Incubation time (minutes)	10 min
Unit	mg/dl
Reagent volume (μl)	300 μl
Sample volume (μl)	3 μl
Calibrator type	Linear
absorbance (A) was Read of the standard and samples at against Blank	

Calculation

$$\text{Serum total cholesterol, (mg/dl)} = \frac{\text{Abs. sample}}{\text{Abs. standard}} \times 200$$
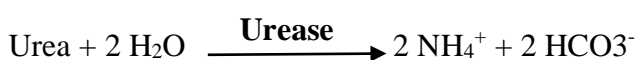
Reference value

Total Cholesterol (Serum/Plasma)	
Recommended values	< 200 mg/dl
Upper limit	200 - 239 mg/dl
High values	≥ 240 mg/dl

3.11.6.2 Determination of serum triglycerides

Serum triglycerides was determined by using enzymatic colorimetric method with (glycerol-3 phosphate-oxidase (GPO)) **(Bucolo et al., 1973).** using a commercially available kits (AMS, Italy).

Principle

The method is based on the hemolysis of serum triglycerides to glycerol and free fatty acid by lipoprotein lipase (LPL). is transformed by glycerolkinase (GK) into glycerol-3-phosphate (G-3-P) which is oxidized by glycerolphaphate oxidase (GPO) into dihydroxyacetone phosphate (DHAP) and hydrogen peroxide. In presence of peroxidase (POD), the hydrogen peroxide oxidizes the chromogen ESPT (4-aminophenazone/N-ethylmethylanilin- propan-sulphonate sodic) to form purple quinoneimine, whose color intensity, measured at 550 nm, is proportional to the concentration of triglycerides in the sample.

$$\text{Triglyceride} + H_2O \xrightarrow{\text{LPL}} \text{Glycerol} + \text{Fatty acids}$$

$$\text{Glycerol} + ATP \xrightarrow{\text{GK}} \text{Glycerol-3-phosphate} + ADP$$

$$\text{Glycerol-3-phosphate} + O_2 \xrightarrow{\text{GPO}} \text{Dihydroxyacetone phosphate} + H_2O_2$$

$$2H_2O_2 + \text{Amminoantipirine} + ESPT \xrightarrow{\text{POD}} \text{Quinoneimine}_{\text{(purple complex)}} + HCl + 4 H_2O$$

Reagents and components

Reagents	Component	Concentration
Reagent-A:	Good Buffer	50 mmol/l
	ESPT	4 mmol/l
	ATP	2 mmol/l
	Mg++	2 mmol/l
	Lipoproteinlipase (LPL)	≥ 1 kU/l
	Glycerol kinase (GK)	≥ 0.4 kU/l
	Glycerolphosphate oxidase (GPO)	≥ 1.5 kU/l
	4- Amminoantipirine	0.5 mmol/l
	Peroxidase (POD)	>1 kU/l
	NaN3	≤ 0.095 g/l
	pH	7.2
Standard (Std.):	Glycerol (triglycerides equivalent)	200 mg/dl
	NaN3	≤ 0.095 g/l

Reagent preparation

Triglycerides single reagent was ready to use.

Assay procedure

About 0.5 ml of serum was transferred to the Chemistry (Autoanalyzer Rayto-chemray240, Hamburg, Germany). to perform the test according to these parameters:

Parameter	Value
Reaction type	End point
Wavelength (nm)	550 nm
Temperature	37 C°
Direction	Increase
Mix and incubation time (minutes)	5 min
Unit	mg/dl
Reagent volume (μl)	300 μl
Sample volume (μl)	3 μl
Calibrator type	Linear
absorbance (A) was read of the standard and samples at against blank	

Calculation

$$\text{Serum triglycerides, (mg/dl)} = \frac{\text{Abs. sample}}{\text{Abs. standard}} \times 200$$

Reference value

Triglycerides (Serum/Plasma)	
Recommended values	< 200 mg/dl
Upper limit	200 - 400 mg/dl
High values	> 400 mg/dl

3.11.6.3 Determination of high-density lipoprotein (HDL-C)

HDL-C was determined by using liquid HDL precipitant for the determination of HDL Cholesterol (precipitation of LDL, very low density lipoprotein (VLDL) and chylomicrons method) **(Grove, 1979).** using a commercially available kits (Diasys, Halzheim, Germany).

Principle

Low density lipoproteins (LDL), very low density lipoproteins (VLDL), and chylomicrons of serum sample are precipitated by adding phosphotungstic acid and magnesium ions. After centrifugation, HDL are in the supernatant. Cholesterol

included in this phase, is measured enzymatically colorimetric method using cholesterol reagent.

Reagents and components

Reagents	Component	Concentration
Reagent	phosphotungstic acid Magnesium chloride	0.55 mmol/L 25 mmol/L
Standard (Std.):	Choesterol standard	200 mg/dl

Reagent preparation

HDL precipitant single reagent was ready to use.

Assay procedure

Precipitation

Add to 200 µL of serum sample, was added to 500 µL of precipitating reagent. Mix well; allow standing for 10 minute at room temperature, and centrifuging for 10 minute at 4000 rpm. After centrifugation, separate the clear supernatant for HDL determination from the precipitate within 1 hour.

HDL determination

Recovery about 0.5 ml of the supernatant for the HDL cholesterol determination was transferred to the Chemistry (Autoanalyzer Rayto-chemray 240, Hamburg, Germany). to perform the test according to these parameters. The cholesterol kit is used for HDL cholesterol determination.

Parameter	Value
Reaction type	End point
Wavelength (nm)	510 nm
Temperature	37 C°
Direction	Increase
Mix and incubation time (minutes)	10 min
Unit	mg/dl
Reagent volume (μl)	300 μl
Sample volume (μl)	3 μl
Calibrator type	Linear
absorbance (A) was read of the standard and samples at against blank	

Calculation

$$\text{Serum HDL, (mg/dl)} = \frac{\triangle A \text{ Abs. sample}}{\triangle A \text{ Abs. standard}} \text{ X } 200$$

Reference value

Serum HDL-C	
Low value	< 40 mg/dl (High risk)
Medium value	40 - 59 mg/dl (moderate risk)
High value	> 60 mg/dl (low risk)

3.11.6.4 Calculation of low-density lipoprotein (LDL)

Low-density lipoprotein (LDL) was estimated from quantitative measurements of total cholesterol, triglycerides and HDL using Friedewald formula (**Friedewald et al., 1972**).

Principle

LDL is most commonly estimated from quantitative measurements of cholesterol, HDL and triglycerides using the empirical relationship of Friedewald formula:

LDL (mg/dl) = Total Cholesterol – (HDL + triglycerides / 5).

The Friedewald equation should not be used when chylomicrons are present, and when serum triglycerides concentration exceeds 400 mg/dl.

3.11.7 Determination of serum total protein

Serum total protein was determined by using colorimetric biuret endpoint method (**Gornall et al., 1949**). using a commercially available kits (AMS, Italy).

Principle

Proteins react with the cupper ions present in the biuret reagent giving in alkaline medium to form a blue-violet colored complex. The intensity of the color formed is directly proportional to the amount of proteins in the sample.

Proteins + Cu++ $\longrightarrow$ Blue violet colored complex.

Reagents and components

Reagents	Component	Concentration
Reagent-A:	K-Na Tartrate	318 mmol/l
	KJ 30	mmol/l
	CuSO4	12 mmol/l
	Sodium hydroxide	600 mmol/l
Standard (Std.):	liquid Stabilized proteic solution	6 g/dl

Reagent preparation

Total Proteins single reagent was ready to use.

Assay procedure

Parameter	Value
Reaction type	End point
Wavelength (nm)	546 nm
Temperature	37 C^o
Direction	Increase
Mix and incubation time (minutes)	5 min
Unit	g/dl
Reagent volume (μl)	300 μl
Sample volume (μl)	6 μl
Calibrator type	Linear
absorbance (A) was read of the standard and samples at against blank	

Calculation

$$\text{Serum total proteins, (g/dl)} = \frac{\text{Abs. sample}}{\text{Abs. standard}} \times 6$$
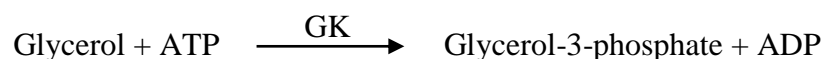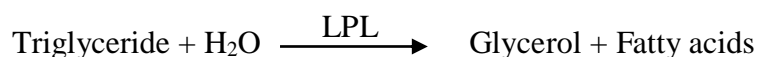
Reference value

Total Proteins (Serum/Plasma)	
Adults:	6 - 6.8 g/dl

3.11.8 Determination of serum albumin

Serum albumin was determined by using colorimetric endpoint with bromocresol green (BCG) method (**Doumas et al., 1949).** using a commercially available kits (AMS, Italy).

Principle

This method was based on the specific binding in citrate buffer albumin in the presence bromocresol green (BCG) at a slightly acid pH produces a color change to form BCG-albumin Complex. The intensity of the color formed is proportional to the concentration of albumin in the sample.

$$\text{BCG+ Albumin} \xrightarrow{\text{pH 4.3}} \text{BCG-albumin complex.}$$

Reagents and components

Reagents	Component	Concentration
Reagent-A:	Citrate buffer	7.5 mmol/l
	BCG	≥ 150 mmol/l
	Sodium azide	0.05%
Standard (Std.):	Albumin	4 g/dl
	Sodium azide	0.05%

Reagent preparation

Serum albumin single reagent was ready to use.

Assay procedure

Parameter	Value
Reaction type	End point
Wavelength (nm)	546 nm
Temperature	37 C°
Direction	Increase
Mix and incubation time (minutes)	3 min
Unit	g/dl
Reagent volume (μl)	400 μl
Sample volume (μl)	2.5 μl
Calibrator type	Linear
absorbance (A) was read of the standard and samples at against blank	

Calculation

$$\text{Serum albumin, (g/dl)} = \frac{\text{Abs. sample}}{\text{Abs. standard}} \times 4$$

Reference value

Albumin (Serum/Plasma)	
Adults:	3.5 – 5.0 g/dl

3.11.9 Determination of serum phosphorus

Serum phosphorus was determined by using phosphomolybdate UV endpoint method with Amonium Molybdate **(Simonsen et al., 1946).** using a commercially available kits (AMS, Italy).

Principle

Inorganic phosphate reacts in acid medium with ammonium molybdate to form a phosphomolybdate complex. The intensity of the color formed is directly proportional to the amount of inorganic phosphate in the sample at absorption 340 nm.

Ammonium molybdate + Nitric acid $\xrightarrow{\text{Phosphorus}}$ Phosphomolybdic complex

Reagents and components

Reagents	Component	Concentration
Reagent-A:	Ammonium molibdate	0.5 mmol/l
	Nitric acid	200 mmo/l
	Surfactant and preservatives	---
Standard (Std.):	Inorganic phosphorus	4 mg/dl

Reagent preparation

Serum phosphorus single reagent was ready to use.

Assay procedure

Parameter	Value
Reaction type	End point
Wavelength (nm)	340 nm
Temperature	37 C^o
Direction	Increase
Mix and incubation time (minutes)	2 min
Unit	mg/dl
Reagent volume (μl)	300 μl
Sample volume (μl)	3 μl
Calibrator type	Linear
absorbance (A) was read of the standard and samples at against blank	

Calculation

$$\text{Serum phosphorus, (mg/dl)} = \frac{\text{Abs. sample}}{\text{Abs. standard}} \times 4$$

Reference value

Phosphorus (Serum/Plasma)	
Adults:	2.3 – 4.7 g/dl

3.11.10 Determination of serum sodium, potassium, total calcium, ionized calcium and chloride

Serum electrolytes parameters (Na^+, K^+, t.Ca, i.Ca^{++} Cl$^-$) were determined by using ion selective electrode (ISE) method **(Oesch et al., 1986).** The instrument analyzer was used Cornley-AFT-500 Electrolyte, (Nanshan industrial), China The instrument performs self-calibration and when the calibration process is correctly performed, the screen of the instrument displays ready status and the serum sample for electrolytes can be measured. Three levels of controls; l, II, III, were analyzed with each run.

Reagent

The reagents were package kit insert in the instrument analyzer by calibration standard solution for used with (ISE).

Components	Reagents Kit	
	CAL-A concentration	CAL-B concentration
K^+	mmol/l 4.0	mmol/l8.0
Na^+	mmol/l140.0	mmol/l110.0
Cl$^-$	mmol/l100.0	mmol/l70.0
Ca^{++}	mmol/l1.25	mmol/l2.5
PH	7.40	7.0

Procedure

○ To be able to perform the tests, the instrument calibrates itself automatically by One and two point calibrations using Cal-A, and Cal-B every 2 hours.

○ Pull the sampler up to the stat position and Press the sample aspiration key the analyzer itself measured automatically.

○ Wait until the printed result appears after about 45 second.

Reference value

Serum Electrolytes	
Sodium(Na^+)	135-145 mEq/L
Potassium (K^+)	3.5-5.3 mEq/L
Total Calcium (T.Ca$^+$)	8.5-10.8 mg/dl
Ionized Calcium (i.Ca^{++})	4.3-5.2 mg/dl
Chloride (Cl$^-$)	95-107 mEq/L

3.12 Limitations of the study

− The continuous and frequent outages of electricity.

− The difficulty of providing the necessary materials and controls for the study due to the siege of Gaza and the frequent closure of the crossings.

− increase of the cost price biochemical material and equipment for tests.

− Compliance with the schedule of dialysis patients who were selected for study within 24 hours and prolong the time of the dialysis session for about 4 hours.

3.13 Data analysis data entry and statistical analyses

Data was collected, summarized, tabulated and statistical analyses were performed using SPSS (Statistical Package for Social Sciences) software version 22. The variables were analyzed using descriptive statistics, Chi-Square test (χ^2), One sample t test, Independent samples t test and Paired t test. Results were presented through tables and Bar charts. The statistically significant differences were recognized when the P values less than < 0.05 at confidence interval 95.0%.

Chapter -4

Result

Chapter -4

Result

4.1 Personal characteristics of the study participants

The results showed that the mean ± standard deviation (SD) of age among the males group were 36.3±11.5 years whereas, it was 33.0±11.8 among females group. However, the t-test statistical analysis showed that there was no statistically significant difference between the study population with respect to mean ± (SD) of age in years (P=0.217).

Table 4.1 summarizes general characteristics of study population. shows the comparative distribution of the study population according to residence & educational level. After using of Chi-Square test (χ^2); there were no statistically differences among the study subjects with respect to residence (P=0.044). In contrast, there were statistically differences among the study subjects with respect to education level (0.001).

In this study all 80 patients who were dialyzed in 32 dialysis machine were selected randomly according to age and duration of hemodialysis for at least 3 months. About 66 (82.5%) of dialysis machine included in this study were new (The machine is new it uses period less than 5 years) whereas the rest number was old machine 14 (17.5%).

Table (4.1): Distribution of study participants by general characteristics

Variables	Males group N=40 (%)	Females group N=40 (%)	Total	χ^2	P-Value
Age Group					
16-27 years	10 12.5%	16 20.0%	26 32.5%	2.051	0.359
28 – 41 years	15 18.8%	12 15.0%	27 33.8%		
42 – 56 years	15 18.8%	12 15.0%	27 33.8%		
Residence					
Gaza	17 21.3%	26 32.5%	43 53.8%	4.073	0.044
North Gaza	23 28.7%	14 17.5%	37 46.3%		
Level of education					
Illiterate	1 1.3%	2 2.5%	3 3.8%	17.975	0.001*
Primary	11 13.8%	5 6.3%	16 %20.0		
preparatory	13 16.3%	2 2.5%	15 18.8%		
Secondary	8 10.0%	23 28.7%	31 38.8%		
Graduate	7 8.8%	8 10.0%	15 18.8%		
Total	**40 50.0%**	**40 50.0%**	**80 %100.0**		

P value by chi-square test, P < 0.05 is statistical significant. * Statistically significant

4.2 The study participant's distribution based on their clinical information by gender

The table 4.2 shows distribution of study participants based on clinical information by gender. After using of Chi-Square test (χ^2); there were a statistically differences among the study subjects with respect neuropathy complication (P=0.048).

Table (4.2): Distribution of study participants based on their clinical information by gender

Variables	Males group N=40 (%)	Females group N=40 (%)	Total	χ^2	P-Value
Family history of renal failure					
Yes	12 57.1%	9 42.9%	21 100%	**0.581**	**0.446**
No	28 47.5%	31 52.5%	59 100%		
Do you have diabetes					
Yes	5 62.5%	3 37.5%	8 100%	**0.556**	**0.456**
No	35 48.6%	37 51.4%	72 100%		
Do you have hypertension					
Yes	34 54.0%	29 46.0%	63 100%	**1.867**	**0.172**
No	6 35.3%	11 64.7%	17 100%		
Do you have retinopathy					
Yes	9 60.0%	6 40.0%	15 100%	**0.738**	**0.390**
No	31 47.7%	34 52.3%	65 100%		
Do you have neuropathy					
Yes	1 14.3%	6 85.7%	7 100%	**3.914**	**0.048***
No	39 53.4%	34 46.6%	73 100%		
Do you have cardiovascular diseases					
Yes	5 62.5%	3 37.5%	8 100%	**0.556**	**0.456**
No	35 48.6%	37 51.4%	72 100%		
Do you have complain of bone disease					
Yes	3 37.5%	5 62.5%	8 100%	**0.556**	**0.456**
No	37 51.4%	35 48.6%	72 100%		
Do you have recurrent infections					
Yes	6 46.2%	7 53.8%	13 100%	**0.092**	**0.762**
No	34 50.7%	33 49.3%	67 100%		
Do you have obesity					
Yes	2 40.0%	3 60.0%	5 100%	**0.213**	**0.644**
No	38 50.7%	37 49.3%	75 100%		
Total	**40 50.0%**	**40 50.0%**	**80 %100.0**		

P value by chi-square test, P < 0.05 is statistical significant. * Statistically significant

4.3 Anthropometric measures, clinical and biochemical parameters of the study population pre-dialysis and post-dialysis by gender

4.3.1 BMI, weight and blood pressure of study population pre-dialysis and post-dialysis by gender

The table 4.3.1 describes the differences in BMI, weight and blood pressure between males and females participating in the study pre-dialysis and post-dialysis. After applying Independent- t test; The means ± (SDs) of Pre-D weight and Post-D weight among males are significantly higher than among females these differences reach a statistically significant (p<0.05).

Table (4.3.1): BMI, weight and blood pressure of study population pre-dialysis and post-dialysis by gender

Variable	Male group N=40 Mean ±SD	Female group N=40 Mean ±SD	t Test	P- value
Pre-D BMI (Kg/m^2)	25.13 ± 6.0	24.21 ± 6.03	0.67	**0.500**
Post-D BMI (Kg/m^2)	24.31 ± 5.93	23.26 ± 5.89	0.79	**0.429**
Pre-D Weight (Kg)	70.69 ± 23.48	59.81 ± 18.11	2.319	**0.023***
Post-D Weight (Kg)	68.42 ± 23.10	57.46 ± 17.70	2.382	**0.020***
Pre-D SBP (mm Hg)	156.45 ± 30.96	147.27 ± 24.06	1.48	**0.143**
Post-D SBP (mm Hg)	150.82 ± 26.77	145.40 ± 27.52	0.89	**0.374**
Pre-D DBP (mm Hg)	86.37 ± 17.30	92.42 ± 18.55	0.15	**0.136**
Post-D DBP (mm Hg)	83.20 ± 15.07	90.37 ± 18.71	1.88	**0.063**

BMI: Body Mass Index , **SBP**: Systolic Blood Pressure , **DBP**: Diastolic Blood Pressure
P value by Independent-t test, P< 0.05 is statistical significant.*Statistically significant

4.3.2 Kidney function test and cystatin C levels of study population pre-dialysis and post-dialysis by gender

Table 4.3.2 illustrates the differences in means ± (SDs) of kidney function tests and Cys-C levels between males and females participating in the study pre-dialysis and post-dialysis. However, there were no statistically differences in the means ± (SDs) of kidney function tests and Cys-C levels among the study population pre and post dialysis (P>0.05) according to Independent- t test.

Table (4.3.2): Kidney function tests and cystatin C levels of study population pre-dialysis and post-dialysis by gender

Variable	Male group N=40 Mean ±SD	Female group N=40 Mean ±SD	t Test	P- value
Pre-D Cys-C (mg/L)	4.10 ± 0.70	4.01 ± 0.89	0.492	**0.624**
Post-D Cys-C (mg/L)	3.08 ± 0.63	2.85 ± 0.71	1.474	**0.145**
Pre-D Urea (mg/dL)	145.0 ± 44.36	135.10 ± 42.64	1.020	**0.311**
Post-D Urea (mg/dL)	55.62 ± 24.95	45.52 ± 20.99	1.959	**0.054**
Pre-D Creatinine (mg/dl)	11.53 ± 3.58	10.53 ± 2.89	1.361	**0.178**
Post-D Creatinine (mg/dl)	4.70 ± 2.22	4.07 ± 1.45	1.507	**0.136**
Pre-D Uric acid (mg/dl)	6.41 ± 1.52	6.32 ± 1.74	0.259	**0.796**
Post-D Uric acid (mg/dl)	3.78 ± 1.64	3.48 ± 1.65	0.807	**0.422**

P value by Independent- t test, P < 0.05 is statistical significant.

4.3.3 Blood glucose, lipid profiles, total protein and albumin levels of study population pre-dialysis and post-dialysis by gender

The table 4.3.3 describes the blood glucose, lipid profiles, total protein and albumin levels between males and females participating in the study pre-dialysis and post-dialysis. The means ± (SDs) of pre-D HDL-C (mg/dl) and Post-D HDL-C (mg/dl) among females were significantly higher than those among males these differences reach a statistically significant (p<0.05). In contrast, the means ± (SDs) of other biochemical parameters were exhibit no changes among males as compared to thus among female. These findings were identified using Independent- t test.

Table (4.3.3): Blood glucose, lipid profiles, total protein and albumin levels of study population pre-dialysis and post-dialysis by gender

Variable	Male group N=40 Mean ±SD	Female group N=40 Mean ±SD	t Test	P- value
Pre-D Glucose (mg/dl)	131.72 ± 49.55	130.0 ± 45.57	0.157	**0.875**
Post-D Glucose (mg/dl)	164.37 ± 49.58	159.92 ± 53.91	0.384	**0.702**
Pre-D Cholesterol (mg/dl)	161.22 ± 45.41	158.82 ± 40.95	0.248	**0.805**
Post-D Cholesterol (mg/dl)	181.47 ± 48.25	185.07 ± 43.43	-0.351	**0.727**
Pre-D Triglycerides (mg/dl)	164.0 ± 73.43	138.27 ± 64.59	1.664	**0.100**
Post-D Triglycerides (mg/dl)	209.70 ± 94.11	183.37 ± 69.34	1.424	**0.158**

Variable	Male group N=40 Mean ±SD	Female group N=40 Mean ±SD	t Test	P- value
Pre-D HDL-C (mg/dl)	40.10 ± 12.41	48.42 ± 15.46	-2.656	**0.010***
Post-D HDL-C (mg/dl)	47.67 ± 12.83	55.50 ± 14.29	-2.576	**0.012***
Pre-D LDL-C (mg/dl)	88.25 ± 45.35	82.75 ± 41.76	0.564	**0.574**
Post-D LDL-C (mg/dl)	91.82 ± 51.19	92.85 ± 44.19	-0.096	**0.924**
Pre-D Total Protein (g/dL)	7.64 ± 0.89	7.28 ± 0.81	1.909	**0.060**
Post-D Total Protein (g/dL)	8.81 ± 1.19	8.35 ± 1.01	1.871	**0.065**
Pre-D Albumin (mg/dl)	4.55 ± 0.59	4.26 ± 0.45	2.449	**0.017***
Post-D Albumin (mg/dl)	4.87 ± 0.59	4.65 ± 0.57	1.633	**0.107**

HDL-C: high density lipoprotein-cholesterol, **LDL-C:** low density lipoprotein-cholesterol

P value by Independent- t test, P < 0.05 is statistical significant. * Statistically significant

4.3.4 Electrolytes parameters and phosphorus levels of study population pre-dialysis and post-dialysis by gender

The table 4.3.4 describes the electrolytes parameters and phosphorus levels between males and females participating in the study pre-dialysis and post-dialysis. However, after using Independent- t test; the means ± (SDs) of electrolytes parameters and phosphorus levels were exhibit no changes among males as compared to thus among female pre and post dialysis (p>0.05).

Table (4.3.4): Electrolytes parameters and phosphorus levels of study population pre-dialysis and post-dialysis by gender

Variable	Male group N=40 Mean ±SD	Female group N=40 Mean ±SD	t Test	P- value
Pre-D Sodium (mEq/L)	137.45 ± 3.74	137.85 ± 3.82	-0.473	**0.638**
Post-D Sodium (mEq/L)	139.70 ± 3.85	138.95 ± 3.0	0.970	**0.335**
Pre-D Potassium (mEq/L)	5.16 ± 0.59	5.0 ± 0.59	1.145	**0.256**
Post-D Potassium (mEq/L)	3.52 ± 0.61	3.46 ± 0.35	0.535	**0.594**
Pre-D Calcium (mg/dl)	7.52 ± 1.17	7.31 ± 1.0	0.848	**0.399**
Post-D Calcium (mg/dl)	8.29 ± 0.80	8.10 ± 0.80	1.010	**0.316**
Pre-D Calcium ionized (mg/dL)	3.72 ± 0.63	3.60 ± 0.51	0.865	**0.390**
Post-D Calcium ionized (mg/dL)	4.12 ± 0.43	4.0 ± 0.42	1.286	**0.202**
Pre-D Chloride (mEq/L)	96.87 ± 3.53	97.60 ± 3.62	-0.906	**0.368**
Post-D Chloride (mEq/L)	94.80 ± 2.51	94.45 ± 2.65	0.606	**0.546**
Pre-D Phosphorus (mg/dl)	5.85 ± 1.56	5.19 ± 1.40	1.982	**0.051**
Post-D Phosphorus (mg/dl)	3.73 ± 1.14	3.29 ± 1.12	1.747	**0.085**

P value by Independent- t test, P < 0.05 is statistical significant.

4.3.5 CBC parameters of study population pre-dialysis and post-dialysis by gender

Table 4.3.5 reveals the means ± (SDs) of CBC parameters of study population pre-dialysis by gender. However, the means ± (SDs) of CBC parameters were exhibit no changes among males as compared to thus among female pre and post dialysis (p>0.05) with respect to Independent- t test.

Table (4.3.5): CBC Parameters of study population pre-dialysis by gender

Variable	Male group N=40 Mean ±SD	Female group N=40 Mean ±SD	t Test	P- value
HGB (g/dl)	8.01 ± 1.19	7.73 ± 0.97	1.169	**0.246**
HCT (%)	23.52 ± 3.86	22.10 ± 4.36	1.535	**0.129**
RBCs (10^6/µl)	2.89 ± 0.46	2.80 ± 0.42	0.898	**0.372**
MCV (fl)	81.40 ± 5.24	81.35 ± 5.95	0.040	**0.968**
MCH (pg)	27.80 ± 2.33	27.77 ± 2.27	0.530	**0.958**
MCHC (g/dL)	34.16 ± 1.71	34.16 ± 1.07	0.008	**0.994**
PLT (10^3/µl)	204.07 ± 80.89	224.20 ± 157.81	-0.718	**0.475**
WBCs (10^3/µl)	5.49 ± 1.52	5.01 ± 1.72	1.311	**0.194**

P value by Independent- t test, P < 0.05 is statistical significant.

4.4 The effect of hemodialysis on anthropometric measures, clinical and biochemical parameters of study population pre-dialysis and post-dialysis

4.4.1 The effect of hemodialysis on BMI, weight and blood pressure of study population pre-dialysis and post-dialysis

The table 4.4.1 illustrates the effect of hemodialysis on BMI, weight and blood pressure. The paired- t test results reported that there were highly statistically significant decreased in BMI and body weight post-dialysis as compared to pre-dialysis (P=0.000). On the other hand, SBP and DBP were not changed among the study population post-dialysis as compared to pre-dialysis (P=0.181 and 0.116 respectively).

Table (4.4.1): The effect of hemodialysis on BMI, weight and blood pressure of study population pre-dialysis and post-dialysis

Variable	Pre-dialysis N=80 Mean ±SD	Post-dialysis N=80 Mean ±SD	Paired t Test	P- value
BMI (Kg/m^2)	24.67 ± 5.99	23.78 ± 5.89	19.08	**0.000****
Weight (Kg)	65.25 ± 21.54	62.94 ± 21.18	18.22	**0.000****
SBP (mm Hg)	151.86 ± 27.93	148.11 ± 27.11	1.348	**0.181**
DBP (mm Hg)	89.40 ± 18.08	86.78 ± 17.26	1.588	**0.116**
*BMI: Body Mass Index , ***SBP:** Systolic Blood Pressure , ***DBP:** Diastolic Blood Pressure				

P value by paired- t test, P < 0.05 is statistical significant. ** Highly statistically significant

4.4.2 The effect of hemodialysis on kidney function tests and cystatin C levels of study population pre-dialysis and post-dialysis

The table 4.4.2 which summarizes the effect of hemodialysis on kidney function tests and Cys-C levels among the study population. However, a highly statistically significance decrease in urea, creatinine, uric acid and Cys-C levels were observed among the study population post-dialysis as compared to pre-dialysis with respect to paired t test analysis (P= 0.000 for all).

Table (4.4.2): The effect of hemodialysis on kidney function tests and cystatin C levels of study population pre-dialysis and post-dialysis

Variable	Pre-dialysis N=80 Mean ±SD	Post-dialysis N=80 Mean ±SD	Paired t Test	P- value
Cys-C (mg/L)	4.05 ± 0.80	2.96 ± 0.68	18.263	**0.000****
Urea (mg/dL)	140.06 ± 43.52	50.57 ± 23.46	20.417	**0.000****
Creatinine (mg/dl)	11.03 ± 3.27	4.39 ± 1.89	24.012	**0.000****
Uric acid (mg/dl)	6.36 ± 1.63	3.63 ± 1.64	14.749	**0.000****

P value by paired- t test, P < 0.05 is statistical significant. ** Highly statistically significant

4.4.3 The effect of hemodialysis on blood glucose, lipid profiles, total protein and albumin levels of study population pre-dialysis and post-dialysis

As it is clear in the table 4.4.3 which summarizes the effect of hemodialysis on blood glucose, lipid profiles, total protein and albumin levels among the study population. However, Paired t test indicated a highly statistically significance increase in blood glucose, cholesterol, triglycerides, HDL-C, LDL-C, total protein and albumin levels were observed among the study population post-dialysis as compared to pre-dialysis (P=0.000 for all).

Table (4.4.3): The effect of hemodialysis on blood glucose, lipid profiles, total protein and albumin levels of study population pre-dialysis and post-dialysis

Variable	Pre-dialysis N=80 Mean ±SD	Post-dialysis N=80 Mean ±SD	Paired t Test	P- value
Glucose (mg/dl)	130.88 ± 47.31	162.15 ± 51.51	-5.961	**0.000****
Cholesterol (mg/dl)	160.02 ± 42.98	183.27 ± 45.65	-10.077	**0.000****
Triglycerides (mg/dl)	151.13 ± 69.92	196.53 ± 83.19	-9.644	**0.000****
HDL-C (mg/dl)	44.26 ± 14.54	51.58 ± 14.06	-9.203	**0.000****
LDL-C (mg/dl)	85.50 ± 43.41	92.33 ± 47.52	-3.10	**0.003***
Total protein (g/dL)	7.46 ± 0.86	8.58 ± 1.12	-10.778	**0.000****
Albumin (mg/dl)	4.41 ± 0.54	4.76 ± 0.59	-7.955	**0.000****
HDL-C: high density lipoprotein-cholesterol **LDL-C:** low density lipoprotein-cholesterol				

P value by paired- t test, P < 0.05 is statistical significant. * Statistically significant.
** Highly statistically significant

4.4.4 The effect of hemodialysis on electrolytes parameters and phosphorus levels of study population pre-dialysis and post-dialysis

As shown in the table 4.4.4 which summarizes the effect of hemodialysis on electrolytes parameters and phosphorus levels among the study population. However, Paired t test reported a highly statistically significance elevated in blood sodium, calcium and calcium ionized while low blood potassium, chloride and phosphorus levels were observed among the study population post-dialysis as compared to pre-dialysis (P=0.000 for all).

Table (4.4.4): The effect of hemodialysis on electrolytes parameters and phosphorus levels of study population pre-dialysis and post-dialysis.

Variable	Pre-dialysis N=80 Mean ±SD	Post-dialysis N=80 Mean ±SD	Paired t Test	P- value
Sodium (mEq/L)	137.65 ± 3.76	139.32 ± 3.45	-3.361	**0.000****
Potassium (mEq/L)	5.08 ± 0.59	3.49 ± 0.49	19.931	**0.000****
Calcium (mg/dl)	7.41 ± 1.09	8.19 ± 0.80	-7.760	**0.000****
Calcium ionized (mg/dl)	3.66 ± 0.58	4.06 ± 0.42	-7.273	**0.000****
Chloride (mEq/L)	97.23 ± 3.57	94.62 ± 2.57	6.950	**0.000****
Phosphorus (mg/dl)	5.52 ± 1.51	3.51 ± 1.14	14.339	**0.000****

P value by paired- t test, P < 0.05 is statistical significant. ** Highly statistically significant

4.5 Hemodialysis adequacy markers and its correlation with each other

4.5.1 The hemodialysis adequacy markers

According to the renal standards document recommendation, all patients stable on three times per a week hemodialysis should shows: a URR ≥ 65% **(National Kidney Foundation (NKF), K/DOQI, 2015).** There was significant Cys-C reduction through high-flux hemodialysis. The CCRR was 26.48 ± 11.05%. This is lower than the small solutes clearance (URR, and CrRR). The URR and CrRR were 62.58 ± 16.34 % and 59.85 ± 12.98 %, respectively. However, there was no significant relation between CCRR, and the small solute clearance.

Table (4.5.1): The Mean ± stander deviation of hemodialysis adequacy markers

Variable	Subjects N=80 Mean ±SD
URR	62.58 ± 16.34%
Kt/V	1.069 ± 0.414
SP-Kt/V	1.061 ± 0.440
CrRR	59.85 ± 12.98%
CCRR	26.48 ± 11.05%
*URR: Urea Reduction Ratio**, *Kt/V:** Treatment Index **,*SP-Kt/V:** Single-Pool Kt/V **, *CrRR:** Creatinine Reduction Ratio, ***CCRR:** Cys-C Reduction Ratio	

4.5.2 The correlation analysis between URR, Kt/V, SP-Kt/V, CrRR and CCRR

Table 4.5.2, illustrates the correlation analysis between URR, Kt/V, SP-Kt/V, CrRR and CCRR. The results showed that there were statistically significant correlations between the CCRR with URR, Kt/V, SP-Kt/V and CrRR ($P < 0.05$). these correlations were weak (r=0.225, 0.306, 0.309, 0.405 respectively). In the other hand, as expected, there were strong correlations between Kt/V and SP-Kt/V (r=0.991, p<0.000) and URR (r=0. 967, p=0.000).

Table (4.5.2): The correlation analysis between URR, Kt/V, SP-Kt/V, CrRR and CCRR

Correlations		URR	KT/V	SP-Kt/V	CrRR	CCRR
URR	**Pearson Correlation**	1	0.967[**]	0.947[**]	0.672[**]	0.225[*]
	P- value		0.000	0.000	0.000	0.045
KT/V	**Pearson Correlation**	0.967[**]	1	0.991[**]	0.759[**]	0.306[**]
	P- value	0.000		0.000	0.000	0.006
SP-Kt/V	**Pearson Correlation**	0.947[**]	0.991[**]	1	0.759[**]	0.309[**]
	P- value	0.000	0.000		0.000	0.005
CrRR	**Pearson Correlation**	0.672[**]	0.759[**]	0.759[**]	1	0.405[**]
	P- value	0.000	0.000	0.000		0.000
CCRR	**Pearson Correlation**	0.225[*]	0.306[**]	0.309[**]	0.405[**]	1
	P- value	0.045	0.006	0.005	0.000	
	Total	80	80	80	80	80

*URR: Urea Reduction Ratio, ***Kt/V:** Treatment Index, ***SP-Kt/V:** Single-Pool Kt/V, ***CrRR:** Creatinine Reduction Ratio, ***CCRR:** Cys-C Reduction Ratio

• Correlation is significant at the P 0.05 level .

4.6 Comparison between Kt/V and SP-Kt/V based on adequacy dose of dialysis

The table 4.6, One sample t test, the results showed that there was adequacy in both Kt/V and SP-Kt/V among study subjects (P=0.006 & 0.006 respectively). On the other hand, both Kt/V and SP-Kt/V have the same adequacy dose of dialysis because they have same P. value.

Table (4.6): Comparison between Kt/V and SP-Kt/V based on adequacy dose of dialysis

Variable	Subjects N=80 Mean ±SD	t Test	P- value
Kt/V	1.069 ± 0.414	2.812	**0.006***
SP-Kt/V	1.061 ± 0.440	2.811	**0.006***
*Kt/V: Treatment Index，***SP-Kt/V:** Single-Pool Kt/V			

P value by one sample t test, P < 0.05 is statistical significant. * statistically significant

4.7 Estimation of Cut-off value for CrRR and CCRR among study population

The table 4.7, One-sample test, the findings showed that the percent adequacy cut-off value for CrRR was 62.75% and 24.03% for CCRR.

Table (4.7): Estimation of Cut-off value for CrRR and CCRR among study population

Variable	Test Value = 62.75% (N=80)			
	t	df	Sig.	Mean Difference
CrRR	1.993	79	0.05	2.894
	Test Value = 24.03% (N=80)			
CCRR	1.987	79	0.05	2.4552
*CrRR: Creatinine Reduction Ratio, ***CCRR:** Cys-C Reduction Ratio				

By One sample t test, and at P = 0.05; the cutoff values are estimated.

4.8: Comparison of URR, CRR, CCRR, Kt/V and SP-Kt/V with clearance standers based on adequacy by gender

The table 4.8, shows comparison of URR, CrRR, CCRR, Kt/V and SP-Kt/V with clearance standers for adequacy and inadequacy by gender. After using of Chi-Square test (χ^2); there were statistically differences among the study subjects with respect to URR, Kt/V and SP-Kt/V (P=0.024, 0.025 and 0.025 respectively). In addition, table 4.8 also indicates that the females group had higher adequacy for all parameters in comparison to the males group.

Table (4.8): Comparison of URR, CRR, CCRR, Kt/V and SP-Kt/V with clearance standers based on adequacy by gender

Variables	Males group N=40 (%)	Females group N=40 (%)	Total	χ^2	P-Value
URR					
Adequacy	18 22.5%	28 35.0%	46 57.5%	**5.115**	**0.024***
Inadequacy	22 27.5%	12 15.0%	34 42.5%		
CrRR					
Adequacy	14 17.5%	18 22.5%	32 40.0%	**0.833**	**0.361**
Inadequacy	26 32.5%	22 27.5%	48 60.0%		
CCRR					
Adequacy	21 26.3%	22 27.5%	43 53.8%	**0.050**	**0.823**
Inadequacy	19 23.8%	18 22.5%	37 46.3%		
Kt/V					
Adequacy	14 17.5%	24 30.0%	38 47.5%	**5.013**	**0.025***
Inadequacy	26 32.5%	16 20.0%	42 52.5%		
SP-Kt/V					
Adequacy	14 17.5%	24 30.0%	38 47.5%	**5.013**	**0.025***
Inadequacy	26 32.5%	16 20.0%	42 52.5%		
Total	**40 50%**	**40 50%**	**80 %100**		
*URR: Urea Reduction Ratio, *Kt/V: Treatment Index, *SP-Kt/V: Single-Pool Kt/V , *CrRR: Creatinine Reduction Ratio, *CCRR: Cys-C Reduction Ratio					

P value by chi-square test, P < 0.05 is statistical significant. * Statistically significant

Figure (4.1): Distribution of dialysis dose clearance parameters based on adequacy

Figure (4.2): Distribution of dialysis dose clearance parameters based on adequacy by gender

Chapter -5

Discussion

Chapter -5

Discussion

As mentioned previously in the present study, 80 patients were dialyzed in 32 dialysis machine, were selected randomly according to age and duration of hemodialysis for at least 3 months. dialysis machines used in this study were new that it uses period was less than 5 years. About 66 (82.5%) of total dialysis machines, whereas the rest number were old machines 14 (17.5%).

5.1 The effect of hemodialysis on BMI, weight and blood pressure

BMI and body weight are predictive indicator of obesity-related diseases such as CVD and Diabetes mellitus (DM). Our findings reported that, BMI and body weight of the study population were decreased significantly after dialysis. So far, quite a few studies have been carried out regarding BMI and HD patients. The majority of the studies in the literature were in the line of our results where they reported that HD patients had lower BMI and body weight after dialysis than pre-dialysis **(Aoyagi, et al., 2001).** It was suggested that the lower BMI indicated malnutrition or cachexia of CRF. Similar results have been found by **Wen, et al., (2007)** where he reported that body weight changes significantly reduced from 57.57±6.78 kg to 55.26±9.92 kg among the study population (p<0.05) after the HD. The decrease in body weight and BMI among the study population after dialysis was attributed to the elimination of excessive body fluids by dialysis machine.

On the other hand, our study showed no change in SBP and DBP among the study population post-dialysis compared to pre-dialysis (P=0.181 and 0.116 respectively). This results were consistent with the main results of the **Walsh's et al., (2005)** systematic review of the effect of HD on blood pressure, anemia, mineral metabolism, and health-related quality of life, where they reported a non-significant reduction in blood pressure of CKD after HD as compared to thus before it. In contrast, these findings are not in accordance with those obtained by **Chan et al., (2012)** study, who found a reduction in systolic blood pressure by the end of HD session.

5.2 The effect of hemodialysis on kidney function tests and cystatin C levels

Increased levels of urea and creatinine excretion in blood due to renal failure made very complication in patients before HD. In renal failure patients, Serum creatinine, urea, uric acid and Cys-C levels are significantly higher than normal range. With respect to the effect of HD on kidney function tests and Cys-C levels among the study population, the current findings observed a highly statistically significance decrease in creatinine, urea, uric acid and Cys-C levels among the study population post-dialysis compared to pre-dialysis (P= 0.000 for all). However, HD showed an effective effect on serum creatinine, urea and uric acid levels which reduces it near to the normal range. The results of this present study were near to the findings of **Khalid, (2015)** who reported that renal failure patients undergoing HD which enacted positive effect on a significant fall in serum creatinine, urea and uric acid levels. Where he presented that all HD patients had serum creatinine below 5.03 1.76 ±and serum urea below 58.26 ± 19.95 after dialysis.

Similar results were also reported by **Draczevski et al., (2011)** who reported that the assessed pre- and post-hemodialysis creatinine and urea levels, obtained reflected a significant reduction in serum levels, indicating HD as an efficient technique. Removal of waste during dialysis also depends upon proper timings of dialysis, patient awareness, and appropriate dialyzer and dietary habits of patients **(Nisha, et al., 2017).** Urea and creatinine levels are important biomarkers as they play a valuable role in diagnosis and follow-up of kidney failure **(Nisha, et al., 2017).**

Cys-C is secreted by nucleated cells, its concentration is not affected by age, sex, diet, inflammation and other factors, which make it an ideal endogenous marker of GFR changes and renal function **(Zhang, et al., (2013).**

In the present study, HD also showed an effective impact on serum Cys-C levels which reduces it near to the normal range. Similar results have been observed by a meta-analysis entitled by clinical evaluation of serum cystatin C and creatinine in patients with CKD **(Zhang, et al., 2013).** The diagnostic sensitivity of Cys-C was higher than that with creatinine, but the diagnostic specificity was lower with Cys-C

(Zhang, et al., 2013). This meta-analysis results showed that there was a good correlation between Cys-C, SCr and the gold-standard measures of GFR. The diagnosis of kidney disease using Cys-C demonstrated modest heterogeneity, but there was no significant heterogeneity in the diagnosis of kidney disease using serum creatinine **(Zhang, et al., 2013).**

In contrast, two studies registered that the serum Cys-C levels were significantly higher in the post-dialysis samples as compared with the pre-dialysis ones **(Krishnamurthy, et al., 2010 and Montini, et al., 2002).** In these studies, the rise in the serum Cys-C following dialysis was attributed to several factors such as the nature of the dialyzing membrane and the composition of the dialyzing fluid **(Krishnamurthy, et al., 2010 and Montini, et al., 2002).**

When dialysis is carried out using low flux membrane, the pore size is smaller than 1.5 nm which does not permit the removal of low molecular weight proteins such as Cys-C. Another factor to be considered is the electro-static interaction between micro-proteins and other plasma proteins adsorbed onto the dialyzer membranes. Cys-C is strongly cationic and the charged nature of the molecule might hinder its filtration **(Krishnamurthy et al., 2010).**

The rise in serum Cys-C could also be attributed to the effect of hemo-concentration which occurs during dialysis. The fall in serum creatinine despite such changes is because of the magnitude of reduction of this metabolite during dialysis. In conclusion, the results of the study have shown that serum Cys-C cannot be used to monitor adequacy of HD. However, it serves as a surrogate marker of the inadequacy of dialysis, more so when low flux membranes are used. Cys-C is considered to be a prognostic biomarker of risk for CVD and death. **(Krishnamurthy et al., 2010).**

5.3 Effect of hemodialysis on blood glucose, lipid profiles, total protein and albumin levels

The present study also showed a highly statistically significance increase in blood glucose, lipid profiles, total protein and albumin levels were observed among the study population post-dialysis compared to pre-dialysis (P=0.000 for all).

Regarding to the effect of HD on plasma glucose, patients with ESRD requiring HD have poor control of their blood glucose. The results of this study suggested that patients who receiving HD had higher glycemic levels post-dialysis as compared to pre-dialysis. This increases may have attributed by researcher to the food intake and juices drinking during HD session.

In addition, it is important to mention that the blood glucose test type carried out this study was random blood glucose because the majority of ESRD patient's intake food and drinks during dialysis session's and it's very difficult to select fasting volunteers.

Similar results were reported by other study done by **Jackson, et al., (2000)** where they found that HD induced hypoglycemia in patients of ESRD with and without diabetes mellitus. later study that was conducted to evaluate the effect of HD on plasma glucose levels among ESRD, but its results were not in agreement with our findings, where it was indicated that the mean of glucose was significantly lower on post- HD as compared with those on pre- HD **(Jin, et al., 2015).**

With concern to the impact of HD on lipid profile (cholesterol, triglycerides, HDL-C, LDL-C), Our findings were agreed with the majority of studies in the literature which studied the impact of HD on lipid profile among CRF patients and showed hyperlipidemia is associated to HD **(Maurya, et al., 2018 and Anjankar et al., 2014).** This hyperlipidemia observed in the current study is because the bad habits of eating and drinking which followed by patients during the period of HD session. On other connected statement, continuous HD patients develop atherogenic serum lipid profile. Total cholesterol, HDL-C, Triglycerides, VLDL-C level was found elevated in regular HD patients as compared to irregular HD patients **(Maurya, et al., 2018).**

In contrast to the present study findings, other study found that serum triglycerides were significantly increased post dialysis as compared to thus pre dialysis, and HDL-C was significantly lowered among patients with ESRD, while the serum cholesterol, LDL-C, VLDL and chylomicron levels were not significantly changed **(Maheshwari et al., 2010).**

Correlation between the albumin levels and hydration status of HD patients has been investigated **(Kubrusly et al., 2012).** That study showed that HD increased total protein and albumin levels, similar result was reported by **Kubrusly et al., (2012**) where they found that post-HD albumin increased in 93.1% of the patients. This increase was clearly related to intradialytic fluid loss, 1.93 kg on average, as was demonstrated through the significant correlation between the pre-HD and post-HD difference of the albumin values and weight. **Colin et al., (2002)** also reported a significant correlation between increased serum albumin and the change of the extracellular volume. **Dumler, (2003)** demonstrated a significant increase of the extracellular volume in a group of patients with albumin < 3.5 g/dL, in relation to the group with higher albumin levels. As same as, **Stolic, et al., (2010)** conducted their study on one hundred and forty patients, 82 (58.6%) male, and 58 (41.4%) female, to find out if there is any correlation between markers of nutrition with the adequacy of HD. Their results found that protein status showed significantly higher values in patients post-HD.

5.4 Effect of hemodialysis on electrolytes parameters and phosphorus levels

As mentioned previously the results of the present findings found a highly statistically significance elevated in blood sodium, calcium and calcium ionized while low blood potassium, chloride and phosphorus levels were observed among the study population post-dialysis as compared to pre-dialysis (P=0.000 for all). Similar results were published by **Wen, et al., (2007)** where they carried out their cross sectional survey on 23 ESRD patients to determine relationship between electrolytes (Ca^{+2}, P^{+3}, Na^{+}, K^{+}, Cl^{-}) and heart rate variability parameters in ESRD patients before and after hemodialysis. After using paired t-test, their results showed that Ca^{+2}, P^{+3}, Na^{+}, K^{+}, Cl^{-} were increased significantly after HD **(Wen, et al., 2007).** All electrolytes in our study except chloride changes pre and post-HD were shown to be statistically significant by paired t test.

Moreover, Similar results were reported by the cross-sectional study that was conducted to evaluate the electrolyte changes (K^{+}, Na^{+}, Ca^{++}, Mg^{++}, Cl^{-}, phosphate) between the pre and post-dialysis phases. Forty patients with ESRD (22 men, 18 women, mean age 44 years) were included in that study. The study found that serum

K$^+$(5.3 ±0.56 vs 3.36 ±0.41 mEq/L, p < 0.001), phosphate (7.19 ± 1.62 vs 3.81 ± 1.02 mg/dL, p <0.001), magnesium (0.87 ± 18 vs 0.75±0.14 mg/dL) were significantly decreased, whereas the Ca^{++} (2.21 ±0.18 vs 2.47 ±0.24 mg/dL, p < 0.001) concentrations was significantly increased after HD compared to pre-dialysis values **(Yetkin, et al., 2000).**

As same as, a study conducted by **Kirschbaum, (2003)** to assess the effect of HD on electrolytes and acid–base parameters. He showed that among both K$^+$ and Cl$^-$ included electrolytes parameters, exhibited statistically significant decreases after HD as compared to before dialysis. The K$^+$ concentration fell by 1.3 mmol/l and all post-dialysis K$^+$ concentrations were below 3.5 mmol/l. The Cl$^-$ concentration fell by 3 mmol/l and all post-dialysis Cl$^-$ concentrations were below 100 mmol/l **(Kirschbaum, 2003).**

5.5 Comparison of URR, CrRR and CCRR with clearance standers

Based on the renal standards document recommendation, all patients stable on three times per a week hemodialysis should shows: a URR ≥ 65% **(National Kidney Foundation (NKF), K/DOQI, 2015).** The findings of the current study showed that there was non-adequacy in urea among study subjects. Based on P value; CCRR was better as adequacy marker than CrRR which in order was better as adequacy marker than URR.

Concerning using CCRR as an adequacy marker, many studies in the literature were researched it. In our study, the CCRR was 26.48 ± 11.05%. In addition, our observations showed a highly statistically significance decrease in Cys-C levels among the study population after high-flux HD. These findings are consisting with the study by **Al-Malki et al., (2009)** who researching the levels of Cys-C in ESRD patients under dialysis and published a statistically significant reduction in Cys-C after high-flux HD. He found that the CCRR was 26.1 ± 11.8%. In contrast, **Thysel et al., (1988)** conducted a study entitled with Cys-C: a new marker of biocompatibility or a good marker for the redistribution of low mw proteins during HD and they reported a relative increase in Cys-C post-HD. This might due to hemoconcentration and slow equilibration of Cys-C between intravascular and extravascular spaces. A one previous study reported a significant

difference between high- and low-flux dialyzers in Cys-C clearance **(Park et al., 2010).** CCRR results were $42.4 \pm 6.3\%$ with high-flux dialyzers and $11.5 \pm 16.2\%$ with low- flux dialyzers, respectively, and this differences reach a statistically significant levels **(Park et al., 2010).** The lower CCRR of 26.48% post-HD in the current study despite very high blood flows may be due to differences in UF rates and the different methods of assay for Cys-C.

Concerning to URR and CrRR, our findings presented that the URR and CrRR were 62.58 ± 16.34 % and 59.85 ± 12.98 % respectively. Similar results were reported in many recent studies, the most famous one is conducted by **Huang et al., (2011)** where he showed that the URR and CRR, and CCRR were $70.2\% \pm 9.0\%$, $64.5\% \pm 8.2\%$ respectively. **Amini, et al., (2011)** published a research suggests that the urea reduction ratio less than 65%, thus our URR value was the $62.58 \pm 16.34\%$.

With regard to Kt/V and SP-Kt/V, our findings showed that Kt/V and SP-Kt/V have the same adequacy dose of dialysis because they have same P value and this results also were reported by **Al Saran, et al., (2010).** The mean Kt/V and SP-Kt/V were calculated to be 1.069 ± 0.414 and 1.061 ± 0.440, respectively. These findings were in the line with previous study reported that the mean of Kt/V was calculated to be 1.2 ± 0.4. However, A Kt/V less than 1.2 as same as our value **(Amini, et al., 2011).**

Furthermore, in the present study CCRR was correlated with URR, Kt/V, SP-Kt/V, CrRR and CCRR. Our results showed that there was a statistically significant correlation between the CCRR with URR, Kt/V, SP-Kt/V and CrRR (P<0.05). These correlations were weak (r=0.225, 0.306, 0.309, 0.405 respectively). In the other hand, as expected, there were strong correlations between Kt/V and SP-Kt/V (r=0.991, p<0.000) and URR (r=0. 967, p=0.000). **Park et al., (2010)** also revealed a weak correlation between CCRR, and URR and Kt/V, SP-Kt/V. In contrast, a study reported that there was no correlation between the CCRR, and the Sp-Kt/V, URR, and CRR. **(Huang, et al., 2011).**

Finally, according to One-sample test, the findings showed that the percent adequacy cut-off value for CrRR is 62.75% and 24.03% for CCRR.

Chapter 6
Conclusion and Recommendation

Chapter -6
Conclusion and recommendation

6.1 Conclusion

1. This study is the first one performing the cystatin c and some biochemical parameters for dialysis adequacy among hemodialysed patients.

2. Neuropathy complications were more common among females as compared to males on hemodialysis.

3. Males have higher means ± (SDs) of Pre-DWeight and Post-DWeight than thus among female.

4. There were no statistically differences of kidney function tests and Cys-C levels among the study population pre and post dialysis.

5. The pre-D HDL-C (mg/dl) and Post-D HDL-C (mg/dl) among female are higher significantly than thus among males.

6. Electrolytes parameters and phosphorus levels were exhibit no changes among the study population pre and post dialysis by gender.

7. BMI and body weight of the study population were decreased significantly post-dialysis as compared to pre-dialysis.

8. A highly statistically significance decrease in urea, creatinine, uric acid and Cys-C levels were observed among the study population after hemodialysis.

9. A highly statistically significance increase in blood glucose, cholesterol, triglycerides, HDL-C, LDL-C, total protein and albumin levels were observed among the study population post-dialysis as compared to pre-dialysis.

10. A highly statistically significance elevated in blood sodium, calcium and calcium ionized while low blood potassium, chloride and phosphorus levels were observed among the study population post-dialysis as compared to pre-dialysis.

11. CCRR was correlated with URR, Kt/V, SP-Kt/V and CrRR, and these correlations were weak. In other hand, as expected, there were strong correlations between URR with Kt/V and SP-Kt/V.

12. Kt/V and SP-Kt/V have the same adequacy dose of dialysis because they have same P value.

13. The percent adequacy cut-off value for CrRR was 62.75% and 24.03% for CCRR, and these values were estimated statistically for our population.

14. Females group had higher adequacy for all adequacy markers in comparison to males group.

6.2 Recommendations

1. Introducing of Cys-C test for hemodialysis patients as a hemodialysis adequacy marker by calculate CCRR in Gaza hospitals is recommended.

2. There is a need to improve the hemodialysis patient's awareness on bad effect of eating practice during hemodialysis session on adequacy markers.

3. Dialysis adequacy markers should be inserted through schedule follow up of hemodialysis patients to help for monitoring of dialysis adequacy.

4. The cut-off values of CrRR and CCRR which estimated for the study population in this study could be used as an adequacy marker reference range for our population.

5. Further research is required to compare the dialysis adequacy markers by types used to the filtration membrane (high-flux and low flux).

6. Further research is required to investigate the cystatin C and other biochemical parameters for dialysis adequacy among fasting hemodialysis patients.

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Appendices

استبانة

اخي الكريم... اختي الكريمة ،،،،،

السلام عليكــم ورحمةالله وبركاته

اضع بين ايديكم استبانة لدراسة علمية وذلك لنيل درجة الماجستير في تخصص التحاليل الطبية، تحت عنوان:

" سيستاتين سي وبعض المعايير البيوكيميائية لقياس كفاءة الغسيل الدموي لدى مرضى الفشل الكلوي المزمن في مستشفى الشفاء ـ محافظة غزة"

ومن متطلبات الدراسة قد صممت هذه الاستبانة لجمع البيانات والمعلومات التي تخص موضوع الدراسة لتقييم مستوى هرمون السيستاتين سي وبعض المعايير البيو كيميائية لدى مرض اعتلال الكلى المزمن الذي يتطلب عملية غسيل الدم (الديال الدموى) من الذكور والاناث والتي أعمارهم فوق 15 عام في قطاع غزة وهدفها المساعدة في تقييم كفاءة الكلى والوقوف على مسببات المرض للحد من مضاعفاته.

علما بان جميع البيانات المقدمة لن تستخدم الا في أغراض البحث العلمي فقط، وسيتم التعامل معها بالسرية والأمانة وحسب الأصول المهنية المعمول بها في البحث العلمي.

ولكم فائق الاحترام والتقدير

شكراً على حسن تعاونكم

الباحث

فواز حسن العجلة

جوال/0599-689600

Appendix (1)

Questionnaire- (HD patients)
Chronic Kidney Disease Flow Sheet

| Request No. | | Room No. | | Instrument No. | | Instrument status. | |

A-Personal information

English Name: .... **Arabic Name:** ....

Sex: ☐ Male   ☐ Female   **Date of birth:** .... / .... / ....   **Age:** ....

Serial Patient No: .... **Tel. No.:** ....

Residence: ☐ Gaza North   ☐ Gaza   ☐ Mid Zone   ☐ Gaza South

Education (years): ....

Employment: ☐ Yes   ☐ No   ☐ ....

Family income per month (NIS):   ☐ <1000   ☐ 1000-2000   ☐ >3000

BMI:.. **Height:**.................. cm   **Weight Pri-D:** .... Kg
  Weight Post-D: .... Kg

B.P.-Pri :Systolic: .... **Diastolic:**.............. | **B.P.-Post : Systolic:** .... **Diastolic:**................

B- Clinical information:

Age at diagnosis KD (years): .... **Duration of KD (years):** ....

When did you start hemodialysis?...

How many times you receive hemodialysis per week?   ☐ Twice   ☐ Three

How many hours per session? ....hours

Type of dialysis membranes: ☐ Low Flux(LF)   ☐ High Flux(HF)

Do you have ?

Family history of renal failure	☐ Yes	☐ No
Diabetes	☐ Yes	☐ No
Hypertension	☐ Yes	☐ No
Retinopathy	☐ Yes	☐ No
Neuropathy	☐ Yes	☐ No
Cardiovascular diseases	☐ Yes	☐ No
complain of bone disease	☐ Yes	☐ No
Recurrent infections	☐ Yes	☐ No
Obesity	☐ Yes	☐ No

Type of drugs intake: ....

Other: ....

اوافق على تعبئة هذا الاستبيان وما يترتب عليه بوضعي الصحي وان جميع البيانات صحيحة

الاسم والتوقيع: ....   التاريخ: ..../............/............م

تعاونكم حسن على شكرا

الباحث/ فواز حسن العجلة

Appendix (2)

Helsinki committee

المجـــلـــس الفلسطيـــني للبحـــث الصحــــي
Palestinian Health Research Council

تعزيز النظام الصحي الفلسطيني من خلال مأسسة استخدام المعلومات البحثية في صنع القرار
Developing the Palestinian health system through institutionalizing the use of information in decision making

Helsinki Committee
For Ethical Approval

Date: 2017/06/05   **Number:** PHRC/HC/215/17

Name: FAWWAZ H. ELIGLAH   الاسم:

We would like to inform you that the committee had discussed the proposal of your study about:

نفيدكم علماً بأن اللجنة قد ناقشت مقترح دراستكم

حول:

Cystatin C and Some Biochemical Parameters for Dialysis Adequacy Among Hemodialysed Patients at Al-Shifa Hospital, Gaza-Governorate.

The committee has decided to approve the above mentioned research. Approval number PHRC/HC/215/17 in its meeting on 2017/06/05

و قد قررت الموافقة على البحث المذكور عاليه

بالرقم والتاريخ المذكوران عاليه

Signature

Member   Member

5/6/2017

Chairman

Genral Conditions:-
1. Valid for 2 years from the date of approval.
2. It is necessary to notify the committee of any change in the approved study protocol.
3. The committee appreciates receiving a copy of your final research when completed.

Specific Conditions:-

Appendix (3)

Permission Letter

دولة فلسطين
وزارة الصحة

السيد : رامي عيد سليمان العبادله   المحترم   التاريخ:18/04/2017

مدير عام بالوزارة/الإدارة العامة لتنمية القوى البشرية – /وزارة الصحة

السلام عليكم ،،،

الموضوع/ الموضوع/ تسهيل مهمة باحث/ فواز العجلة

التفاصيل //
بخصوص الموضوع أعلاه، يرجي تسهيل مهمة الباحث/ **فواز حسن العجلة**
الملتحق ببرنامج ماجستير العلوم الحياتية- تخصص تحاليل طبية – الجامعة الإسلامية بغزة في إجراء بحث بعنوان:-
**"قياس مستوى سيستاتين سي وبعض المعايير البيوكيميائية لدى مرضى الفشل الكلوي المزمن في مستشفى الشفاء-
محافظة غزة"**
حيث الباحث بحاجة لتعبئة استبانه وجزء من عينة دم سحبت لأغراض تشخيصية من عدد من مرضى الفشل الكلوي المترددين على
قسم غسيل الكلى في مستشفيات وزارة الصحة وعينة ضابطة ممن لا يعانون من هذا المرض.
نأمل توجيهاتكم لذوي الاختصاص بضرورة الحصول على الموافقة المستنيرة من المرضى الذين يوافقون على المشاركة في البحث
ومن ثم تمكين الباحث من تطبيق أدوات الدراسة، وفق الأسس التي يتم بها التعامل في مثل هذا النوع من العينات وعلى مسؤولة
الباحث، بما لا يتعارض مع مصلحة العمل وضمن أخلاقيات البحث العلمي ، دون تحمل الوزارة أي أعباء .

محمد ابراهيم محمد السرساوي
/الإدارة العامة لتنمية القوى البشرية -

التحويلات		
■ مدحت عباس خضر حسن (مدير عام بالوزارة)	← حسن محمد خليل حافظ اللوح(مدير مستشفى)	إجراءاتكم بالخصوص()
■ عبد اللطيف محمد محمد الحاج(مدير عام بالوزارة)	← مدحت عباس خضر حسن (مدير عام بالوزارة)	إجراءاتكم بالخصوص()
■ مدحت عباس خضر حسن (مدير عام بالوزارة)	← رافت حامد يوسف حمدونه(مدير دائرة)	إجراءاتكم بالخصوص()
■ عبد اللطيف محمد محمد الحاج(مدير عام بالوزارة)	← عميد عوني فوزي مشتهى(مدير دائرة)	للإفادة()
■ ()	← عبد اللطيف محمد محمد الحاج(مدير عام بالوزارة)	إجراءاتكم بالخصوص()
■ حسن محمد خليل حافظ اللوح(مدير مستشفى)	← محمد عبدالرحيم احمد زقوت(طبيب مسجل مساعد / ممارس عام)	إجراءاتكم بالخصوص()
■ مدحت عباس خضر حسن (مدير عام بالوزارة)	← كايد احمد عارف احمد(مدير)	إجراءاتكم بالخصوص()
■ محمد ابراهيم محمد السرساوي(مدير دائرة)	← ()	للإفادة()

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Appendix (4)

Permission letter

بسم الله الرحمن الرحيم

الجامعة الإسلامية بغزة

The Islamic University of Gaza

مكتب نائب الرئيس للبحث العلمي والدراسات العليا   هاتف داخلي: 1150

الرقم: ج س غ/35/   Ref:
التاريخ: 04-06-2017   Date:

الأخ الفاضل/ المدير العام لمستشفى الوفاء للتأهيل الطبي والجراحات التخصصية   حفظه الله،،،

السلام عليكم ورحمة الله وبركاته،

الموضوع/ تسهيل مهمة طالب ماجستير

تهـديكم شـئون البحـث العلمـي والدراسـات العليـا أعطـر تحياتهـا، وترجـو مـن سـيادتكم التكـرم بتسـهيل مهمـة الطالـب/ فـواز حسـن محمـد العجلـة، بـرقم جـامعي 120130593 المسـجل فـي برنامج الماجسـتير بكليـة العلـوم قسـم العلـوم الحياتيـة/ تحاليـل طبيـة وذلـك بهـدف تطبيق أدوات الدراسة والحصول علي المعلومات التي تساعده في إعداد الرسالة، والمعنونة ب

سيستاتين سي وبعض المعايير البيوكيميائية لقياس كفاءة الغسيل الدموي لدى مرضى الفشل الكلوي المزمن في مستشفى الشفاء محافظة غزة

Cystatin C and Some Biochemical Parameters for Dialysis Adequacy Among Hemodialysed Patients at Al-Shifa Hospital, Gaza-Governorate

والله ولي التوفيق،،،

نائب الرئيس لشئون البحث العلمي والدراسات العليا

أ.د. عبدالرؤوف علي المناعمة

صورة إلى:-
❖ الملف.

+97082644800

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