Ministry of Higher Education
And Scientific Research
University of Baghdad
College of Dentistry

Oral findings, IgA, IL-6, CRP and kidney function markers in saliva of patients with chronic kidney disease

A thesis
Submitted to the council of the college of Dentistry at University of Baghdad in partial fulfillment of the requirement for the degree of Master of Science in Oral Medicine

By
Ethar Kareem Salim
(B.D.S)

Supervised by
Assist. Professor
Dr. Ameena Ryhan Diajil
B.D.S, M.Sc, Ph.D. (Oral Medicine)

2018 A.D. 1440 A.H.

Certification of the Supervisor

I certify that this thesis entitled "**Oral Findings, IgA, IL-6, CRP and kidney function markers in saliva of patients with chronic kidney disease**" was prepared by **Ethar Kareem Salim** under my supervision at the College of Dentistry/ University of Baghdad in partial fulfilment of the requirements for the degree of Master of Science in Oral Medicine.

Signature
Assist. Professor
Dr. Ameena Ryhan Diajil
B.D.S, M.Sc., Ph.D. (Oral Medicine)
(The supervisor)

Certification of the examination committee and the dean

We, the members of the examining committee, certify that after reading the thesis and examining the student **"Ether Kareem Salim"** in its contents, it is adequate for the award of the Degree of Master of Science in Oral Medicine.

Signature
Professor
Dr. Raad M. Helmi
B.D.S., M.Sc., Oral Med. Certified-USA
(Chairman of Examination Committee)

Signature
Professor
Dr. Taghreed F. Zaidan
B.D.S., M.Sc., Ph.D.
(Oral Medicine)
(Member)

Signature
Professor
Dr. Fawaz D. Al-Aswad
B.D.S., M.Sc., Ph.D.
(Oral Medicine)
(Member)

Approved by the council of the College of Dentistry/University of Baghdad.

Signature
Professor
Dr. Hussain F. Al-Huwaizi
B.D.S, M.Sc., Ph.D.
Dean of the College of Dentistry
University of Baghdad

Dedication

To my parents. . .

For their love, support, sacrifice, guidance and everything they have done since I was born.

To my husband. . .

For his love, care, support, encouragement and patience.

To my sunshine, my little angel, my precious son . . .
"Mohammed"

I dedicate this thesis. . .

With love

Dr. Ithar Kareem

Acknowledgment

Praise be to Allah who has given me the health and power to complete this work.

Deep thanks to **Prof. Dr. Hussain F. Al- Huwaizi** Dean of the College of Dentistry-University of Baghdad for his support to accomplish this study.

All thanks to **Prof. Dr. Nidhal H. Ghaib** Assistant Dean for Scientific Affairs and Students of the College of Dentistry-University of Baghdad for her supporting to post graduate students and studies.

My special thank is offered to Prof. **Dr. Jamal Noori**, the head of the oral diagnosis department.

I want to express my heartfelt gratitude and appreciation to my supervisor **Asst. Prof. Dr. Ameena R. Diajil** for her scientific guidance, valuable advice, help and encouragement during the period of this study wishing her long life and continuous progress.

Special thanks to my seniors at oral medicine department/ college of dentistry (**Prof. Dr. Taghreed F. Zaidan; Prof. Dr. Fawaz D. Al-Aswad**) for their effort in providing guidance and updating information throughout the study.

I would like to extent my thanks to all doctors and staff in Al-Kindey dialysis center.

Special thanks to all patients for their cooperation despite their suffering.

My thanks to all the staff of Al- Nadear clinical laboratory.

I would like to thank **Dr. Mohammed Ghalib** for continuous help in biostatistics analysis.

My special thanks to **Dr. Rabab kubba, Dr. Zainab Isam** for their help.

Finally, I would like to express my gratitude to my brother (Ahmed) and my sisters (Hanan and Arwa) who followed my progress and would have been most proud of me.

Abstract

Background

Chronic kidney disease is a gradual loss of kidney function over a period of months or years with diabetes and hypertension as the leading cause. Chronic kidney disease is divided into five stages. The last stage is named end stage renal disease which is become fatal in the absence of replacement therapy.

The chronic inflammation observed in chronic kidney disease is associated with increased serum levels of acute-phase protein reactants, such as C-reactive protein, and a variety of immune-inflammatory mediators, such as cytokines. Interleukin-1, interleukin-6 and tumor necrosis factor α are among the proinflammatory cytokines that have been related to the pathophysiology of kidney disease. There is an evidence of immune activation at the early stages of chronic kidney disease in the adult people. An association between the levels of C-reactive protein and proinflammatory cytokines, particularly Interleukin-6, has also been detected.

Chronic kidney disease is one of the systemic diseases that can affect the salivary content. Saliva can indicate creatinine and urea levels in chronic kidney disease patients which are the parameters generally measured in blood samples.

Aims of the study

This study aim to determine the oral findings, salivary flow rate, salivary PH and to study of Immunoglobulin A, Interleukin-6, C- reactive protein and kidney function markers (creatinine and urea) in saliva of patients with chronic kidney disease on hemodialysis, those on conservative treatment and compared with control subjects.

Subjects, materials and methods

Ninety subjects were included in this study, divided into three groups: 30 patients with chronic kidney disease on hemodialysis for at least 6 months ago; 30 patients with chronic kidney disease on conservative treatment and 30 healthy control participants. Oral examination was done for each participant with the oral manifestations were recorded. Saliva was collected and salivary flow rate was calculated milliliters per minutes and pH was measured by digital pH meter.

Secretory Immunoglobulin A, Interleukin-6 and C- reactive protein in saliva samples was measured by enzyme linked immunosorbent assay ELISA.

Creatinine level was estimated in saliva samples by colorimetric method. And salivary urea level was measured by Roche - Cobas C 111 analyzer automatically.

Results

Dry mouth, uremic fetor and taste change were the most common oral findings in chronic kidney disease patients on hemodialysis and on conservative treatment. Pale oral mucosa, aphthus ulceration, gingival enlargement, burning sensation and angular cheilitis also seen. No significant differences were found in oral manifestations between the two patients groups.

Salivary flow rate was lower in both patients groups compared to control subjects. Regarding salivary PH, a significant higher salivary PH in both chronic kidney disease patients on hemodialysis and on conservative treatment compared to control subjects.

Salivary immunoglobulin A level was higher in both chronic kidney disease patients on hemodialysis and those on conservative treatment compared to control subjects, although statistically non-significant.

There was a significant increase in salivary Interleukin-6 and C-reactive protein in both patients groups compared to control group. A significant positive correlation was found between salivary Interleukin-6 and C-reactive protein in chronic kidney disease patients on hemodialysis (r= 0.781, p=0.00), those on conservative treatment (r= 0.840, p= 0.00) and in control group (r= 0.816, p= 0.00).

Also, there was a significant increase in salivary creatinine and urea levels in both chronic kidney disease patients on hemodialysis and those on conservative treatment compared to control group. Regarding to serum creatinine and urea level, no significant difference was seen between the two patients groups. A significant positive correlation between salivary creatinine and serum creatinine in chronic kidney disease patients on hemodialysis (r= 0.770, p= 0.00) and those on conservative treatment (r= 0.932, p= 0.00). Also, a significant positive correlation between salivary urea and blood urea in chronic kidney disease patients on hemodialysis (r= 0.860. p= 0.00) and those on conservative treatment (r= 0.858, p= 0.00).

Conclusions

Oral manifestations are common in chronic kidney disease patients. Salivary flow rate is lower in chronic kidney disease patients compared to control subjects. Salivary PH, immunoglobulin A, Interleukin-6, C-reactive protein, creatinine and urea levels is higher in chronic kidney disease patients compared to control subjects.

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List of abbreviations

ACE inhibitors	**Angiotensin converting enzyme inhibitors**
ACR	**Albumin creatinine ratio**
AKI	**Acute kidney injury**
ANOVA	**Analysis of variance**
APD	**Automated peritoneal dialysis**
ARBs	**Angiotensin II receptor blockers**
AV	**Arteriovenous**
BUN	**Blood urea nitrogen**
C.C	**Contingency coefficient**
CAPD	**Continuous ambulatory peritoneal dialysis**
CCPD	**Continuous cycling peritoneal dialysis**
CKD	**Chronic kidney disease**
COX-2	**Cyclooxygenase-2**
CRP	**C-reactive protein**
DDAVP	**1-Deamino-8-D-Arginine Vasopressins**
ddH_2O	**double distilled water**
eGFR	**Estimated glomerular filtration rate**
ELISA	**Enzyme-linked immunesorbent assay**
ESRD	**End stage renal disease**
GFR	**Glomerular filtration rate**
GLDH	**Glutamate dehydrogenase**
Hb	**Hemoglobin concentration**
HIV	**Human immunodeficiency virus**
IgA	**Immunoglobulin A**
IgG	**Immunoglobulin G**

IgM	**Immunoglobulin M**
IL-1β	**Interleukin- 1β**
IL-6	**Interleukin- 6**
KDIGO	**Kidney Disease Improving Global Outcome**
KDOQI	**Kidney Disease Outcomes Quality Initiative**
MD	**Mean difference**
NSAIDs	**Non-steroidal anti-inflammatory drugs**
OD	**Optical density**
PCR	**Protein creatinine ratio**
PTH	**Parathyroid hormone**
SD	**Standard deviation**
SE	**Standard error**
S-IgA	**Secretory immunoglobulin A**
s-VLPD	**Very-low protein diet supplemented with amino acids and ketoacids**
TNF-α	**Tumor necrosis factor- α**

INTRODUCTION

Introduction

Chronic kidney disease (CKD) is a progressive reduction in kidney function (Venktapathy *et al*., 2014), with the prevalence and incidence are growing worldwide with diabetes and hypertension as the leading cause (Levey *et al*., 2005).

Chronic kidney disease is classified into five stages according to the level of proteinuria and kidney function which is measured by the estimated glomerular filtration rate (eGFR) which is derived from age, gender, race and serum creatinine concentration (Levin *et al*., 2013). Patients develop End-Stage Renal Disease (ESRD) once bilateral deterioration of nephrons pass the point of compensation therefore; dialysis therapy and renal transplantation are life-saving procedures in these patients (Dağ *et al*., 2010). Although renal transplantation is the preferred method of treatment for patients with ESRD, the majority of patients are placed on dialysis either while awaiting transplantation or as their only treatment (Fenton *et al*., 1997).

In hemodialysis, urea and other low molecular weight substances diffuse during interchange from the patient's blood across an extra-corporal filtering/dialysis membrane into an electrolyte and pH balanced dialysis solution (Craig, 2008). The frequency and duration of dialysis are related to residual kidney function, protein intake, body size and tolerance to fluid elimination. Typically, patient undergoes hemodialysis three times per week, with each treatment session about three to four hours on standard dialysis units (Glick and Feagans, 2015).

In studies of patients with kidney disease, up to 90% were found to have oral findings of uremia. Some of the presenting signs in renal patients were an ammonia-like taste and smell, gingivitis, stomatitis, reduced salivary flow, xerostomia, and parotitis (Glick and Feagans, 2015).

Diminished erythropoietin and the resultant anemia lead to paleness of the oral mucosa. Impairment of platelet function is occur during uremia (Boccardo *et al*., 2004). This situation combined with the heparin use and other anticoagulants in hemodialysis, lead patients to become prone to ecchymosis, petechiae, and hemorrhages in the oral cavity (Seraj *et al*., 2011).

Radiographic changes of the jaw bones in CKD are common and occur as a result of renal osteodystrophy which are the spectrum of histologic alterations that occur in bone architecture of CKD patients (Rai *et al*., 2016).

Saliva is considered as a filtrate of the blood where different molecules pass through transcellular or paracellular routes (passive intracellular diffusion and active transport or extracellular ultrafiltration respectively) into saliva. As a result, saliva is equivalent to serum, therefore reflecting the physiological state of the body (Bagalad *et al*., 2017).

Numerous systemic diseases have been reported to cause marked and identifiable alterations in salivary secretion. CKD is one of these systemic diseases that can affect the contents of salivary secretion. Saliva can indicate creatinine and urea levels in patients with CKD which are the parameters generally measured in blood samples. Measurement of salivary creatinine and urea in patients with CKD offers several advantages that have been attributed to the saliva use as a diagnostic fluid (Lasisi *et al*., 2016).

It has been reported that Immunoglobulin levels, serum IgG isotypes, and both IgA and IgM production are normal in patients on dialysis (Eleftheriadis *et al*., 2007).

The cytokines that are produced during inflammatory episodes, and that participate in them, are stimulators to produce acute phase proteins.

These inflammation related cytokines include interleukin-1β (IL-1β), interleukin-6 (IL-6), tumour necrosis factor-α (TNF-α), interferon-γ, transforming growth factor-β, with the most important ones are macrophages and monocytes at sites of inflammation. IL-6 is the main stimulator of the most acute phase proteins production (Gabey, 2006).

C-reactive protein (CRP) is an acute phase protein synthesized in the liver and secreted into the bloodstream during an inflammatory process, mostly in response to IL-6 signaling and, to a lesser extent, IL-1β and other pro-inflammatory cytokines (Schmit and Vincent, 2008).

Inflammatory response can reflect an underlying systemic disease. Chronic kidney disease patients demonstrate inflammatory pathways activation, which is accompanied by increased inflammatory markers like cytokines. The increased cytokines levels such as IL-6 and TNF-α have been shown in ESRD. Moreover, these inflammatory markers act as toxins that predict deterioration of kidney function (Khozeymeh *et al*., 2016). In oral cavity, these cytokines play an important role in the inflammatory response in periodontium (Nibali *et al*., 2012).

Aims of the study:

1-To study oral findings that are associated with chronic kidney disease.

2-To assess salivary flow rate and salivary PH in patients with chronic kidney disease on hemodialysis, those on conservative treatment and compared with healthy control.

3-To measure immunoglobulin A, interleukin-6 and C- reactive protein in saliva of patients with chronic kidney disease on hemodialysis, those on conservative treatment.

4-To study the kidney function markers (urea, creatinine) in saliva of patients with chronic kidney disease on hemodialysis, those on conservative treatment.

CHAPTER ONE

REVIEW OF LITERATURE

Review of Literature

1.1. Kidney structure

According to Glick and Feagans (2015), the kidneys are bean-shaped organs located in the retroperitoneum at the level of the waist. Each adult kidney weighs about 160 g and measures 10–15 cm in length. Coronal sectioning of the kidney reveals two separate regions: an outer region called cortex and an inner region known as the medulla (Figure 1-1 A). Structures that are located at the corticomedullary junction extend into the renal hilum and are named papillae. Each papilla is surrounded by a minor calyx that collectively communicates with the major calyces to form the kidney pelvis. The kidney pelvis collects urine flowing from the papillae and passes it to the bladder via the ureters. Vascular flow to the kidneys is supplied by the renal artery, which branches directly from the aorta. This artery subdivides into segmental branches to supply the upper, middle, and lower parts of the kidney. Further subdivisions account for the arteriole-capillary venous network or vas recta. The renal venous drainage is provided by a series of veins leading to the renal vein and lastly to the inferior vena cava.

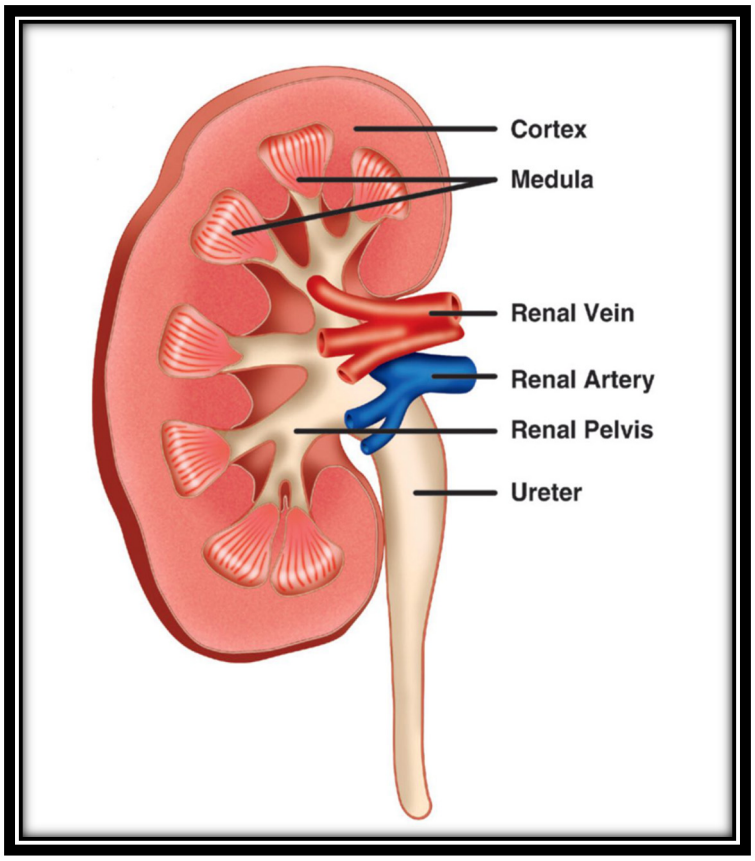


Figure (1-1 A): structure of kidney (Glick and Feagans, 2015)

The kidney's functional unit is the nephron (Figure 1-1 B), and each kidney is made up of nearly one million nephrons. Each nephron consists of Bowman's capsule, which surrounds the glomerular capillary bed; the proximal convoluted tubule; the loop of Henle; and the distal convoluted tubule, which empties into the collecting ducts. The glomerulus is a unique network of capillaries that is suspended between afferent and efferent arterioles enclosed within Bowman's capsule and that serves as a filtering funnel for waste (Glick and Feagans, 2015).

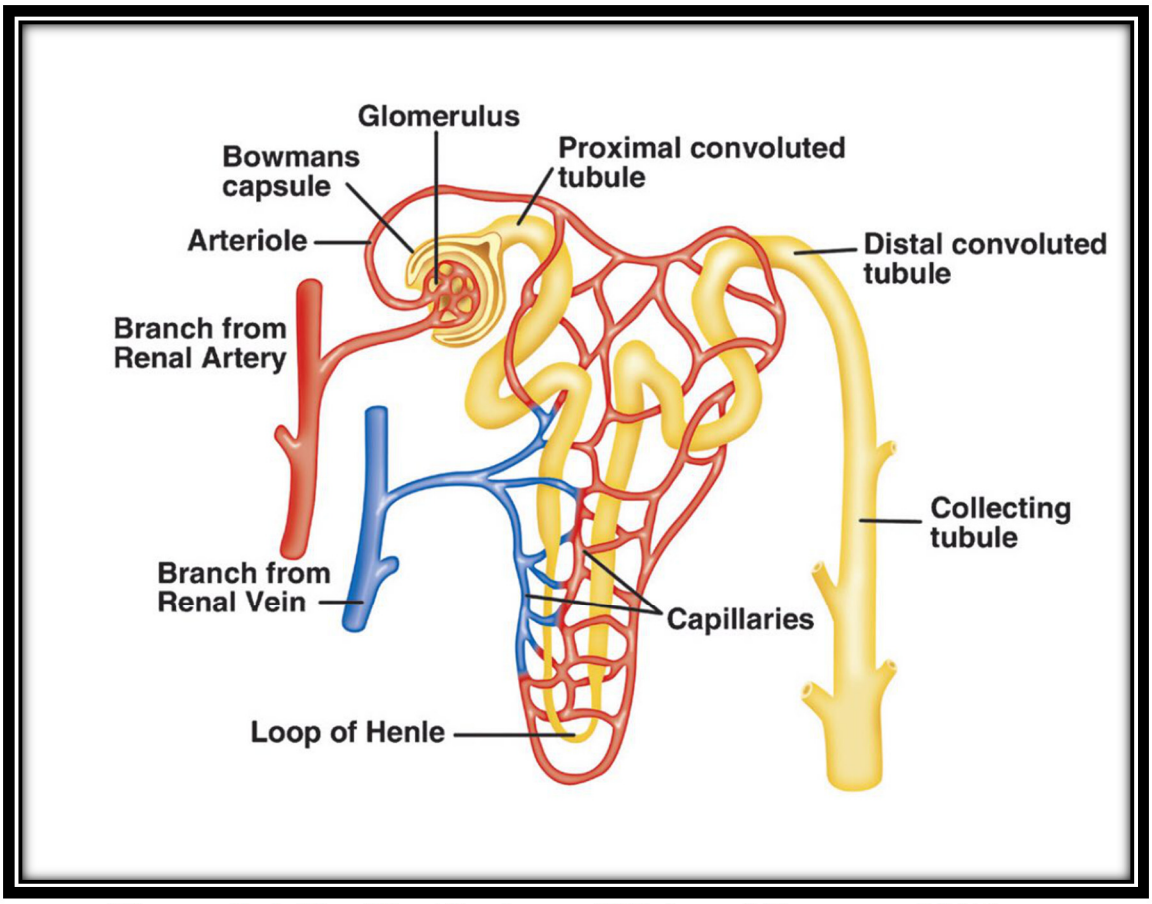


Figure (1-1 B): The Nephron (Glick and Feagans, 2015)

1.2. Kidney functions

According to Walker *et al*., (2015), the functions of kidney as following:

1-The kidneys perform an important role in many metabolic breakdown products excretion, including ammonia, urea and creatinine from protein, and uric acid from nucleic acids, drugs and toxins.

2-They also regulate fluid and electrolyte balance. This is accomplished by making large volumes of an ultrafiltrate of plasma (120 mL/min, 170 L/day) at the glomerulus, and selectively reabsorbing substances of this ultrafiltrate at points along the nephron. The filtration and reabsorption rates are controlled by many hormonal and haemodynamic signals.

3-The kidneys also regulate acid–base homeostasis, calcium and phosphate homeostasis, vitamin D metabolism and red blood cells production. Fibroblast-like cells that lie in the renal cortex interstitium are

responsible for erythropoietin production, which in turn is required for red blood cells production. Erythropoietin synthesis is regulated by oxygen tension; hypoxia and anemia increase production, whereas hyperoxia and polycythemia inhibit it. Erythropoietin production failure plays an important role in the pathogenesis of anemia in CKD.

4-They are important in regulating blood pressure. Renin is secreted from the juxtaglomerular apparatus in response to reduced afferent arteriolar pressure, stimulation of sympathetic nerves, and changes in sodium content of fluid in the distal convoluted tubule at the macula densa, and is the first step in the generation of angiotensin II and aldosterone release, which in turn regulate systemic vasoconstriction and extracellular volume.

1.3. Kidney diseases classification

Kidney diseases are worldwide the 12th main cause of dying and 17th cause for deteriorated life years (Glassock *et al*., 2017). they are classified into two groups depending on the duration of the disease including acute kidney injury which is a sudden insufficiency of renal function (Mehta *et al*., 2007; Rewa and Bagshaw, 2014) and chronic renal disease that is a gradual impairment of kidney function for years (Jha *et al*., 2013; hill *et al*.,2016).

1.3.1. Acute kidney injury

Acute kidney injury (AKI), previously named as acute renal failure, is characterized by a sudden impairment of kidney function. Many studies have found that AKI is associated with an increased mortality and poor outcomes regardless of patient characteristics and the condition in which injury occurs (Hertzberg *et al*., 2017).

Acute kidney injury is common in hospitalized patients, particularly in critically ill patients. In most of the patients, recovery of the renal

function is usually occur; however, many patients remain on dialysis or are left with severe renal insufficiency (Koza, 2016).

1.3.2. Chronic kidney disease

Chronic kidney disease has been defined as kidney damage or an estimated GFR (eGFR) less than 60ml/min/1.73m^2 persisting for three months or more regardless of the cause (Johnson *et al*., 2015).

The kidney disease improving global outcome (KDIGO) classification system recommends that kidney function be estimated by glomerular filtration rate (GFR) calculation from serum creatinine level through a special equation (Matsushita *et al*., 2012).

More than two million worldwide patients with CKD Progress to end stage renal disease and need dialysis or renal transplantation (Hu and coresh, 2017).

1.3.2.1. Glomerular filtration rate

Glomerular filtration rate is the best indicator of kidney function. In chronic kidney diseases, the rate of decline in GFR is a criterion for staging of it and GFR equations are proposed for clinical assessment of kidney function (Levin *et al*., 2013).

Glomerular filtration rate cannot be measured directly but instead, it can be calculated from clearance measurement (measured GFR) or from serum concentration of endogenous filtration markers (estimated GFR) (Levey and Inker, 2016).

The Cockcroft and Gault equation is the most commonly used GFR equation, due to its simple mathematical formula (Lascano and Poggio, 2010). This equation assess creatinine clearance from gender, age, body weight and serum creatinine (Cockcroft and Gault, 1976).

Male Creatinine clearance (ml/min) = ([140 − age] × weight)/ (72 × SCr)

Female Creatinine clearance (ml/min) = Ccr male * 0.85.

Adjustment factor for female (0.85) based on assumption of 15% lower creatinine production due to lower muscle mass (Canaday *et al*., 1984; Johnson *et al*., 2015).

1.3.2.2. Staging of chronic kidney disease:

Kidney Disease Outcome Quality Initiative (KDOQI) suggested a CKD staging system depended on estimated GFR. The original staging ranged from stage 1 CKD known as normal kidney function with other signs of kidney damage to stage 5 CKD known as end stage renal disease or kidney failure (Levey *et al*., 2002).

Kidney disease Improving Global Outcome (KDIGO) lately updated the staging system of CKD. This late staging system is depended on the cause of CKD category, GFR category and albuminuria category (CGA).

The cause category is depended on the association of systemic disease with CKD. The GFR categories admit the necessity to subdivide stage 3 into G3a (eGFR 45_59) and G3b (eGFR 30-44). The albuminuria categories is depended on the existence and seriousness of albuminuria.

This updated classification helped to recognize prognosis of CKD as shown in table 1-1 (Levin *et al*., 2013).

Table (1-1): Kidney disease improving global outcome (KDIGO) staging and prognosis of CKD 2012 (Levin *et al*., 2013).

			Albuminuria categories detailing		
			A1	A2	A3
			normal to mildly increased	Moderately increased	Severely increased
			30 mg/g< 3 mg/mmol<	30-300mg/g 3-30mg/mmol	300mg/g> 30mg/mmol>
G1	Normal or high	90≥			
G2	Mildly reduced	60-89			
G3a	Mildly to moderately reduced	45-59			
G3b	Moderately to severely reduced	30-44			
G4	Severely reduced	15-29			
G5	Kidney failure	15<			

GFR (ml/min/1.72m²) categories detailing

Green: low risk (if no other markers of kidney disease, no CKD)

Yellow: moderately increased risk

Orange: high risk

Red: very high risk

1.3.2.3. End stage renal disease

End stage renal disease is known as an irreversible decline in renal function which is become fatal in the absence of replacement therapy (dialysis or transplantation). End stage renal disease included under stage 5 of the national kidney foundation kidney disease outcome quality initiative (KDIGO) classification of chronic kidney disease with an estimated GFR below 15 ml/min/1.73m² body surface area. Decline in or loss of renal function lead to fluid retention, bone metabolism disorder, anemia, protein-energy malnutrition and dyslipidemia (Abbasi *et al*., 2010).

The most common causes of ESRD are diabetes mellitus, hypertension, chronic glomerulonephritis, autoimmune diseases and uropathy (Hamid *et al*., 2006; Seethalakshmi *et al*., 2014).

1.3.2.4. Epidemiology

Chronic kidney disease is a worldwide public health burden (couser *et al*., 2011). On a global level, 735.000 deaths resulted from CKD in 2010 up from 400.000 deaths in 1990 (Lozano, 2012). More than 100 countries (more than one billion population) have no provision for dialysis or renal transplantation and therefore, more than one million individual die from ESRD yearly (Hamer and EL Nahas, 2006; Levey *et al*., 2007; Lozano, 2012).

Worldwide, More than 2 million people are alive on replacement therapy, but this number represents only 10% of people who need treatment actually. The majority of the 2 million people who receive replacement therapy for renal failure are treated in five countries (Germany, United States, Japan, Italy and Brazil). These countries make up 12% of the world population. In about 100 developing countries that represent more than 50% of the world population, only 20% are received treatment (couser *et al*., 2011).

The number of renal failure cases will increase in developing countries such as India and China where the number of elderly population are increasing (Jha *et al*., 2013).

1.9 to 2.3 million Canadian people diagnosed as CKD. During 1999-2004, the centers for disease control and prevention in United States found that 16.8% of adults at the age of 20 years and older have CKD (CDC, 2007). During 2011 till 2014, the prevalence of CKD stage 1-5 (not involving ESRD) in the US was assessed at 14.8% with stage 3 being the most predominant stage. In 2014, 120.688 new cases of ESRD was recorded (1.1 % increase as compared with 2013). At the end of 2014, A total of 678.383 persons were treated for ESRD (up 3.5% from 2013) (Sarhan *et al*., 2015).

United Kingdom estimates that 8.8% of the people of Great Britain and Northern Ireland affected by CKD (CDC, 2007). Annually, the average incidence of ESRD in UK and Europe is 120.000 and 135.000 respectively (steenkamp *et al*., 2011).

In India, the incidence rate of ESRD is up to 229 per million population (pmp) (Singa *et al*., 2013). Prevalence rate in Saudi Arabia is 5.7% (Alsuwaida *et al*., 2010).

6% of the population in Australia have CKD and in sub-Saharan Africa, the prevalence rates range from 3% to 19% based on CKD stages (Arogundade and Barsoum, 2008; Alsuwaida *et al*., 2010).

People have ESRD represent a small part of the total CKD patients. For example, in US, the total prevalence of CKD (26 million) is 50 times higher than the patients receive treatment with dialysis or a kidney transplantation (collins *et al*., 2014). The cost of replacement therapy (dialysis or kidney transplantation) ranging from 15.000 $ in sub-Saharan.

Africa to over 80.000 $ in the US (Collins *et al*., 2014; Arogundade and Barsoum, 2008; Swanepoel *et al*., 2013).

During searching and reading, no previous study of the epidemiology and prevalence of CKD in Iraq was found.

1.3.2.5. Pathophysiology of chronic kidney disease

The pathophysiology of CKD includes two groups of mechanisms of kidney damage:

1- Intiating mechanisms specified to the underlying cause for example, genetic defects in kidney evolution, deposition of immune complex in particular types of glomerulonephritis, or exposure to toxin in specific diseases of the kidney tubulointerstitium.

2- Progressive mechanisms including hyperfiltration and hypertrophy of the residual functional nephrons that are a common sequel after long term reduction in kidney mass regardless of underlying cause.

The responses to nephron number reduction are mediated by cytokines, vasoactive hormones and growth factors. Lastly, these short term adaptation of hyperfiltration and hypertrophy turn into maladaptive as the increased flow and pressure inside nephron predispose to deformation of glomerular structure, abnormal podocyte function and disturbance of the filtration barrier causing sclerosis and decline of residual nephrons.

Increased activity of renin-angiotensin system (RAS) inside kidney share in both the initial adaptive hyperfilration and the sequent maladaptive hypertrophy and sclerosis.

This process clarify why a renal mass reduction may cause a progressive drop in kidney function over years (Kasper and Harrison, 2015).

1.3.2.6. Causes and risk factors of chronic kidney disease

Chronic kidney disease is caused by conditions that destroy kidney structure and function. According to Innes and Maxwell (2016), the common causes are diabetes mellitus (20-40%), interstitial diseases (20-30%), glomerular diseases (10-20%), hypertension (5-20%), systemic inflammatory diseases (5-10%), renovascular disease (5%), congenital and hereditary factors (5%) and unknown etiology (5-20%).

Older age, family history of kidney disease, low birth weight, African- American race are important risk factors for CKD. Furthermore, obesity, smoking, diabetes mellitus and hypertension can also lead to renal disease. Uncontrolled hypertensive and/or diabetic patients can easily progress to ESRD patients. Alcohol consumption, exposure to heavy metal in addition to the use of analgesic drugs also associated with CKD development (Kasancioglu, 2013).

Acute kidney injury (AKI) has been involved as a risk factor for CKD development in the new observational studies (Rifkin *et al*., 2012).

1.3.2.7. Clinical features of chronic kidney disease

Most patients are asymptomatic till GFR reach below 30 ml/min/1.73 m² (stage 4 or stage 5) and some patients can remain asymptomatic with GFR values much lower than this. Nocturia is an early symptom due to lack of concentrating ability and increased osmotic load per nephron, but this is nonspecific.

When GFR reach below 15-20 ml/min/1.73m², signs and symptoms are common and can influence all body systems. Its include tiredness or breathlessness which may be related to renal anemia, weight loss, anorexia, pruritus, nausea and vomiting. With further decline in kidney function, patients may suffer hiccoughs, experience unusually deep breathing associated with metabolic acidosis (kussmaul's breathing) and

develop muscular twitching, fits, drowsiness and coma (Walker *et al*., 2015).

Fluid retention "odema", bruising easily, brown line pigmentation of nail, peripheral neuropathy "restless legs", loss of libido, renal bone disease "renal osteodysrophy" also seen in advanced CKD (Walker *et al*., 2015).

Renal osteodystrophy results from disturbance in calcium, phosphorus, or vitamin D metabolism and increased parathyroid activity. Calcium absorption by the intestine is reduced early in Chronic renal failure because the kidneys cannot convert vitamin D to its active form (1,25 dihydroxycholecalciferol). There is a corresponding phosphate retention, which eventually leads to decreased serum calcium levels, as calcium phosphate is maintained within normal levels in healthy subjects. This condition is associated with compensatory hyperactivity of the parathyroid gland, leading to increased urinary excretion of phosphates, decreased urine calcium excretion, and increased calcium release from bone (Glick and Feagans, 2015).

1.3.2.8. Oral manifestations

Chronic kidney disease (CKD) like many other systemic diseases, has associated oral manifestations arising from the disease itself or the effects of treatment or both. Consequently, untreated oral lesions may worsen the clinical presentation and prognosis (Wahid *et al*., 2013).

Oral lesions are commonly due to restricted diets, malnutrition, immunosuppression, mouth neglect and the effects of medications and uremic toxins on the oral tissues (Proctor *et al*., 2005).

Chronic kidney disease patients on hemodialysis have also been found to be associated with reduced dental visits which further worsen the oral care (Xie *et al*., 2014).

In studies of renal patients, up to 90% were found to have oral manifestations of uremia. Some of the presenting signs were an ammonia-like smell and taste, gingivitis, stomatitis, xerostomia, and parotitis (Glick and Feagans, 2015).

Reduced erythropoietin and the consequent anemia lead to pallor of the oral mucosa. Platelets dysfunction is occur during uremia (Boccardo et al., 2004). This situation, combined with the heparin use and other anticoagulants in hemodialysis, leads patients to become predisposed to petechiae, ecchymosis, and hemorrhages in the oral cavity (Seraj *et al*., 2011).

Stomatitis, mucositis and glossitis can cause pain and inflammation of the tongue and oral mucosa. Dysgeusia, altered taste sensations, as well as candidiasis and bacterial infections can develop due to the underlying kidney disease (Thomas, 2008). Candidiasis is seen as patients unable to fight infections. Candidiasis is more common in transplant patients because of generalized immunosuppression (Klassen and Krasko, 2002; Olivas-Escárcega *et al*., 2008).

A common oral manifestation of CRD is the sensation of a dry mouth, which may be caused by restricted fluid intake (necessary to accommodate the reduced excretory capability of the kidney), adverse effects of medications, and the low salivary flow rate (Proctor et al., 2005; Gupta and Gupta, 2015). Salivary swelling can be seen occasionally (Glick and Feagans, 2015).

Chronic kidney disease Patients also suffer from odorous breath (uremic breath) and sensations of metallic tastes in the mouth. Uremic fetor occurs as a result of a high salivary urea concentration, which is converted to ammonia (De la Rosa-García *et al*., 2006).

Gingival inflammation has been reported to be due to accumulation of plaque and poor oral hygiene (Olivas-Escárcega *et al*., 2008).

Attention has been given to general medical care, prolonged hospitalization, and hypoplastic teeth as causes of high plaque scores in those patients (Martins *et al*., 2008). However, the frequency of gingival inflammation is low (Al Nowaiser *et al*., 2003; Lucas and Roberts, 2005) because the immunosuppression and uremia associated with kidney disease change the inflammatory response to bacterial plaques in gingival tissue (Nunn *et al*., 2000). Pallor caused by anemia can also cover inflammatory signs of the gingiva (Lucas and Roberts, 2005).

Another manifestation of CKD is gingival enlargement secondary to medications or transplantation. Gingival enlargement specially affects the labial interdental papillae. The unpleasant appearance of gingival enlargement has psychological impacts on the patient, interferes with the normal oral function, speech, and oral hygiene, as well as results in delayed or ectopic eruption. Good oral hygiene is necessary to reduce the inflammation associated with gingival enlargement (Al Nowaiser *et al*., 2003; Chabria *et al*., 2003; Lucas and Roberts, 2005).

Calculus has a significant effect on gingival and periodontal disease incidence. Children with CKD demonstrate an elevated level of calculus (Martins *et al*., 2008). Elevated salivary pH, decreased salivary magnesium, and increased salivary urea and phosphorus lead to precipitation of calcium-phosphorus and calcium oxalate, and thus, calculus formation (Davidovich *et al*., 2009).

An acute rise in the urea level may result in uremic stomatitis, which may manifest as an erythemopultaceous form characterized by red mucosa covered with a thick exudate and a pseudomembrane or as an ulcerative form characterized by frank ulcerations with redness and a pultaceous coat. In all reported cases, these intraoral lesions have been associated with urea levels more than 150 mg/dl and disappear spontaneously when treatment results in a reduced urea level. Although

its exact cause is unknown, uremic stomatitis can be considered as a chemical burn or as a tissue resistance loss and inability to withstand normal and traumatic influences (Glick and Feagans, 2015).

White plaques named “uremic frost” and occasionally seen on the skin can be seen intraorally, although rarely. This uremic frost results from residual urea crystals left on the epithelial surfaces after perspiration evaporates or as a result of decreased salivary flow (Glick and Feagans, 2015).

Disruptions during the histo differentiation, apposition, and mineralization stages of tooth development result in tooth structure anomalies (Mc Donald *et al*., 2011). In children with kidney disease, incidence rates of enamel hypoplasia range from 31% - 83% depending on the ethnic, racial, nutritional, and socio-economic statuses of the child’s family/parent and the type of examination or classification system (Koch *et al*., 1999; Nunn *et al*., 2000; Lucas and Roberts, 2005; Ibarra-Santana et al., 2007; Nakhjavani and Bayramy, 2007; Olivas-Escárcega *et al*., 2008). Enamel hypoplasia of the deciduous and permanent dentition has been observed (Koch *et al*., 1999). Enamel hypoplasia in the form of white or brown discoloration of primary teeth is commonly seen in young children with early-onset kidney disease (Koch *et al*., 1999; Al Nowaiser *et al*., 2003).

Calcium, phosphorus, or vitamin D metabolism can be disturbed in children with CKD during the first months of childhood. Koch et al. reported enamel defects of the primary teeth, especially canine hypoplasia, in 22% of the studied children (Koch *et al*., 1999). Narrowing or calcification of the tooth pulp chamber (Galili *et al*., 1991) and delayed eruption of the permanent dentition (Martins *et al*., 2008) have been reported in children with kidney disease.

In patients with kidney disease, the risk of caries formation is raised by bad oral hygiene and a carbohydrate-rich diet (necessary to minimize the renal workload), in addition to disease-related debilitation, hypoplastic enamel, low salivary flow rate, and long-term drug use (Al Nowaiser *et al*., 2003; Martins *et al*., 2008). Nevertheless, the dental caries incidence appears to be low in these patients, because of the presence of highly buffered and alkaline saliva due to elevated urea and phosphate concentrations (Lucas and Roberts, 2005; Nakhjavani and Bayramy, 2007; Martins *et al*., 2008).

Tooth erosion due to regurgitation associated with dialysis also may be found (Glick and Feagans, 2015).

Manifestations of renal osteodystrophy and compensatory hyperparathyroidism include decreased trabeculation, demineralization and a "ground-glass" appearance of the bone, loss of lamina dura, decreased cortical bone thickness, radiolucent giant cell lesions, maxillary brown tumors, metastatic soft tissue calcification and enlargement of the skeletal base. Patients have an increased risk of jaw fracture due to trauma or oral surgery (Proctor *et al*., 2005).

Other dental findings include malocclusion, tooth mobility, pulp stone, enamel hypoplasia and abnormal bone healing after dental extraction. Radiographically, osteodystrophy appears as a failure of the lamina dura to resorb and the deposition of sclerotic bone around the socket (Klassen and Krasko, 2002; Proctor *et al*., 2005). Children may show brown discoloration of the teeth due to the underlying uremia and oral iron supplements (Martins *et al*., 2008).

1.3.2.9. Diagnosis of chronic kidney disease

1.3.2.9.1. History and physical examination

Particular aspects of the history that are germane to kidney disease involve a history of diabetic mellitus, hypertension which can lead to CKD or more commonly be a result of CKD, abnormal urinalysis and problems with pregnancy (such as preeclampsia or abortion). An accurate medication history should be taken, these involve analgesics, non-steroidal anti-inflammatory drugs, cyclooxygenase-2 (COX-2) inhibitors, antiretroviral agents, antimicrobials, proton pump inhibitors, chemotherapeutic agents, phosphate-containing bowel cathartics and lithium. Family history of kidney disease with determination of manifestations in other organ systems such as visual, auditory and integumentary, may facilitate the diagnosis of a heritable type of CKD, for example Alport or Fabry disease, cystinosis. The physical examination should focus on blood pressure and target organ damage from elevated blood pressure (Kasper and Harrison, 2015).

1.3.2.9.2. Serum Chemistry

In the presence of renal impairment, alteration in homeostasis are seen in serum chemistry. Chloride, sodium, blood urea nitrogen (BUN), creatinine, glucose, carbon dioxide, phosphate, potassium, and calcium levels provide a helpful tool to assess the degree of renal dysfunction and disease progression. Serum creatinine and BUN are often important markers to the GFR. Both of these substances are nitrogenous waste products of protein metabolism that are excreted in the urine in normal manner, but they may raise to toxic levels in the presence of renal impairment. With advancing renal dysfunction, a characteristic profile of change including elevation in serum creatinine, BUN, and phosphate, in contrast to low levels of serum calcium (Glick and Feagans, 2015).

Hemoglobin concentration (Hb), iron, vitamin B12 and folate should also be estimated (Kasper and Harrison, 2015).

1.3.2.9.3. Assessment of proteinuria

Dipstick test of urine and urine culture are important (Arm *et al*., 1986). Proteinuria consider as important diagnostic and prognostic marker, and its presence means a higher risk for both kidney disease progression and cardiovascular complications (Matsushita *et al*., 2010). Kidney disease improving global outcome (KDIGO) recommend that the preferred method of proteinuria assessment is by measuring of the urinary albumin creatinine ratio (ACR) using an early morning urine sample (Levin *et al*., 2013). But, it is important to know that some patients will excrete proteins other than albumin and a urine protein creatinine ratio (PCR) may be more helpful in certain conditions (Methven *et al*., 2011).

A 24-hour urine collection may be useful because protein excretion more than 300 mg may be an indication for requiring angiotensin converting enzyme inhibitors (ACE inhibitors) or angiotensin II receptor blockers (ARBs) therapy (Kasper and Harrison, 2015).

The laboratory findings in progressive kidney disease are summarized in table 1-2.

Table (1-2): Laboratory changes in progressive kidney disease (Glick and Feagans, 2015)

Laboratory test	Normal range	Level in symptomatic renal failure
Glomerular filtration rate	90-120 ml/min/1.73m²	**Less than 15 ml/min**
Serum creatinine	0.6-1.20 mg/dl	**More than 5 mg/dl**
Creatinine clearance	85-125 ml/min(female) 97-140 ml/min (male)	**10-60 ml/min (moderate failure) Less than 15 ml/min (severe failure)**
Blood urea nitrogen	8-18 mg/dl	**More than 50 mg/dl**
Serum calcium	8.5-10.5 mg/dl	**depressed**
Serum potassium	3.8-5 mEq/l	**Elevated**
Serum phosphate	2.5-4.5 mg/dl	**Elevated**

1.3.2.9.4. Kidney imaging

Kidney imaging with ultrasound is helpful in diagnosis. Small kidneys with decreased cortical thickness showing an increased echogenicity, scaring or multiple cysts suggest a chronicity. In some cases, imaging with magnetic resonance, computed tomography or angiography may be advandageous, taking into consideration the risks of contrast media (Johnson *et al*., 2015).

1.3.2.9.5. Biopsy

Attempting to take biopsy from small kidneys is associated with risk and even if a biopsy is taken, histological evaluation may show nonspecific chronic scaring rather than diagnostic characteristic that explain the cause of kidney impairment (Johnson *et al*., 2015).

1.3.2.10. Management of chronic kidney disease

Management of CKD should be aimed at delaying the progression of the disease and minimizing the risk of cardiovascular disease, preventing and treating coexisting conditions, and preparing the patient for renal-replacement therapy (van Dijk and Boner, 2003).

1.3.2.10.1. Conservative treatment

Ricardo *et al*. (2013) clarified that adherence to a healthy lifestyle, including low body mass index, no smoking habits, high physical activity and dietary quality was associated with lower risk of all-cause mortality in people with CKD.

A long list of diet components must be coordinated and monitored in CKD. These typically include calories, protein (type as well as amount), potassium, sodium, calcium, phosphorus, and fluid (in food as well as in liquids). In diabetic patients, carbohydrate will be also monitored. In children, growth parameters become an added focus (Beto *et al*., 2016).

Many studies manifest that diet rich in fruits, vegetables, cereals, whole grain, fish, fibers and polyunsaturated fatty acids but low in saturated fatty acids is advantageous for CKD patients. Also, a very-low protein diet supplemented with amino acids and ketoacids (s-VLPD) was found to be safe and beneficial for patients with CKD, especially at stages 4–5; due to the fact that it corrects proteinuria, hemoglobin and blood pressure and therefore it may prolong not only the dialysis-free period but also the survival rate of patients (Rysz *et al*., 2017).

Treatment with an angiotensin receptor blocker (ARB) or an angiotensin converting enzyme (ACE) inhibitor is guaranteed, with the medication adjusted to achieve a blood pressure below 130/80 mm Hg; reduction of blood pressure to this level delays the rate of decline of the GFR even in patients with advanced stages of chronic kidney disease.

The thiazide diuretic that the patient is taking should be changed by a loop diuretic; if the targeted blood pressure is not reached, a calcium-channel blocker, a beta-blocker, or both should be added. Statin and aspirin are recommended for treatment of hyperlipidemia to reduce the likelihood of cardiovascular disease. The current guidelines of the Kidney Disease Outcomes Quality Initiative (KDOQI) recommend a target hemoglobin concentration of 11 to 12 g /dl. Iron deficiency should routinely be assessed and treated. Serum phosphate levels should be monitored, and if it is higher than 4.6 mg/dl (1.5 mmol/L), a phosphate binder should be given. A low-dose active vitamin D analogue will help to control secondary hyperparathyroidism. A bicarbonate concentration less than 20 mmol/L and systemic acidemia should be treated with sodium bicarbonate (Abboud *et al*., 2010).

1.3.2.10.2. Renal replacement Therapy

End stage renal disease is fatal without renal replacement therapy, which can be provided by either dialysis (hemodialysis or peritoneal dialysis) or by kidney transplantation (Riegden, 2003).

There are no clear guidelines for determining when renal replacement therapy should start. Most nephrologists base their decisions on the individual patient's capability to workfull time, the presence of peripheral neuropathy, and the presence of other signs of clinical deterioration or uremic symptoms. Most nephrologists will begin dialysis when the GFR is below 15 ml/min/1.73 m^2 and the patient is exhibiting uremic symptoms (Glick and Feagans, 2015).

1.3.2.10.2.1. Renal transplantation

Renal transplantation is the treatment of choice for patients with ESRD, as it raises the survival of the recipients and improves their life quality, as compared to long-term dialysis treatment. As the number of patients that need a kidney transplantation rapidly increases, whereas the

supply of organs available for transplantation remains stable or even decreases in some countries, the prolonged time spent on the waiting list for transplantation is nowadays an essential problem for most of patients and specially for those who develop severe complications of ESRD or for those who are not able to undergo dialysis treatment (Kousoulas *et al*., 2013).

Kidney Disease improving global outcome (KDIGO) recommends that living donor preventive renal transplantation in adults be considered when GFR is less than 20 ml/min/1.73m² and there is evidence of progressive and irreversible CKD for more than 6-12 months (Levin *et al*., 2013).

1.3.2.10.2.2. Dialysis

Although renal transplantation is the preferred method of treatment for ESRD patients, most patients are placed on dialysis either while awaiting transplantation or as their only therapy (Fenton *et al*., 1997). There are two main techniques of dialysis: hemodialysis and peritoneal dialysis. Each follows the same basic principle of diffusion of solutes and water from the plasma to the dialysis solution in response to a concentration or pressure gradient (Glick and Feagans, 2015).

A. Hemodialysis

Hemodialysis is the elimination of nitrogenous and toxic products of metabolism from the blood by means of a hemodialyzer system. Interchange occurs between the patient's plasma and dialysate (the electrolyte composition of which mimics that of extracellular fluid) across a semipermeable membrane that allows uremic toxins to diffuse out of the plasma while retaining the formed elements and protein composition of blood. Dialysis does not provide the same degree of health as normal kidney function provides because there is no resorptive ability in the dialysis membrane; therefore, benificial nutrients are

wasted, and potentially toxic molecules are retained (Glick and Feagans, 2015).

The duration and frequency of dialysis treatments are related to residual renal function, body size, protein intake, and tolerance to fluid removal. The typical patient undergoes hemodialysis three times per week, with each treatment lasting approximately three to four hours on standard dialysis units and slightly less time on high efficiency or high-flux dialysis units. There are three main types of vascular access for maintenance hemodialysis: primary arteriovenous (AV) fistula, synthetic AV graft, and double-lumen, cuffed tunneled catheters (Glick and Feagans, 2015).

Routine hemodialysis requires anticoagulation with heparin to prevent clotting in the extracorporeal circuit (Ward, 1995).

Several complications, including muscle cramps, hypotension, disequilibrium syndrome, dyspnea, nausea and vomiting with varying degrees of incidence may occur to the patient during hemodialysis, and nurses require to be familiar with the details of these complications (Asgari et al., 2017). Hemodialysis increases the risk of viral transmission such as hepatitis B, hepatitis C and human immunodeficiency virus (HIV) (Pol, 1995).

Hypotension resulting from depletion of fluid is a common complication of hemodialysis and occurs in up to 30% of dialysis sittings. Cerebrovascular accidents, angina, myocardial infarction and fatal dysrhythmias are less common but serious sequelae of hemodialysis and most commonly present during or immediately following dialysis (Glick and Feagans, 2015).

B. **Peritoneal dialysis**

Peritoneal dialysis is now a widely accepted, effective form of renal replacement therapy for ESRD patients. There are different methods of performing peritoneal dialysis, which are in general divided into continuous cycling peritoneal dialysis (CCPD), also referred to as automated peritoneal dialysis (APD), using the cycler, and continuous ambulatory peritoneal dialysis (CAPD), performed without a machine such as, patients performing the entire therapy manually (Chaudry and Golper, 2015). It includes filling the peritoneal cavity with a dextrose-containing solution and using the peritoneal membrane as a filter to eliminate toxins, regulate electrolytes and remove volume. Historically these patients would not be able to begin with peritoneal dialysis until at least 2 weeks after placement of a peritoneal catheter in order to avoid complications such as abdominal cavity or pericatheter leaks (Naljayan *et al*., 2018).

Peritoneal dialysis has many advantages including greater preservation of residual renal function, greater ease of technique to master, early survival advantage, and superior cost effectiveness compared to hemodialysis. Greater preservation of residual renal function is important as it leads to a better technique survival by enhancing peritoneal dialysis adequacy and ultrafiltration capacity**.** Despite these advantages, patients on peritoneal dialysis remains poor and cardiovascular events continue to be the leading cause of dying in peritoneal dialysis patients (Cho *et al*., 2014).

1.3.2.11. Dental management of patients with chronic kidney disease

The dental management of patients with CKD is complicated by some systemic outcomes of CKD, particularly, liability to bleeding, anemia, and cardiovascular or endocrine disease, but with the employ of

well supervised treatment protocols, the dental management of patients with CKD can be safe and effective (proctor *et al*., 2005).

Before treatment, Consultation with the patient's nephrologist for new laboratory tests and discussion of antibiotic prophylaxis in presence of prior infective endocarditis (Glick and Feagans, 2015).

Lately, periodontal disease has been looked at as a CKD marker. Periodontal pathogens cause both local infection and bacteremia, eliciting local and systemic inflammatory responses. Periodontal disease is associated with the acute phase reactant C-reactive protein (CRP), a main risk factor for CKD. Nonsurgical periodontal treatment has shown to improve periodontal health, endothelial function, and levels of CRP and other inflammatory markers (Glick and Feagans, 2015).

In patients with chronic systemic uremia or nephrotic syndrome, changes of the cellular immunity and malnutrition due to protein-restricted diet adherence lead to immunodeficiency. These patients are liable to bacterial infection and have a diminished ability to produce antibodies (Bagga and Mantan, 2005; Davidovich *et al*., 2005; Glick and Feagans, 2015).Oral diseases and dental procedures create bacteremia, which may lead to morbidity and potential mortality in patients with kidney failure or on dialysis. Oral ulcers, carious teeth, plaque and calculus can be points of entry for microorganisms into the bloodstream. Antibiotic prophylaxis, typically with vancomycin, has been recommended before invasive dental procedures (Gudapati *et al*., 2002; Naylor and Fredericks, 1996; Nunn *et al*., 2000), although this recommendation is contrary to guidelines of the British Society for Antimicrobial Chemotherapy (Proctor *et al*., 2005). Klassen and Krasko (2002) have stated that good oral health reduces the risk of oral infection and subsequently, the risk of endocarditis, septicemia or enteritis at the site of vascular dialysis access.

Presently, there are no clear guidelines for the appropriateness of antibiotic prophylaxis for bacteremia-producing dental procedures in CKD patients. The mild dental infection should be treated with caution. Good home oral care complemented with aggressive in-office oral health maintenance should be employed to lower the risk of dentally induced infections. Patients undergoing dialysis are exposed to numerous transfusions and kidney failure-related immunosuppression; therefore, they are at greater risks of infection by HIV and hepatitis types B and C (Gudapati *et al*., 2002).

In addition, bacterial endocarditis has been reported as unusual but serious complication in the dental management of patients undergoing hemodialysis. They are considered moderate-risk patients and the prescription of a prophylactic antibiotic is particularly important. On the other hand, patients undergoing peritoneal dialysis do not need prophylaxis with antibiotics (Hamid *et al*., 2006).

Excessive bleeding and anemia are the two main hematologic problems that most commonly affect patients with uremia and kidney failure. Bleeding tendencies in these patients are attributed to a combination of quantitative and qualitative platelet defects, increased prostacyclin activity, intrinsic coagulation defects, and capillary fragility. This bleeding tendency magnified in the presence of uremia. Hemorrhagic episodes in the gingiva are not uncommon. Ulcerations and petechial or purpural lesions may be seen throughout the oral mucosa. Bruising after trauma is common, and hematoma formation should be expected after alveolectomy or periodontal surgery. Adjunctive hemostatic measures should be considered for patients who are at risk. 1-Deamino-8-D-Arginine Vasopressins (DDAVP), the synthetic analogue of the antidiuretic hormone vasopressin, has been shown to be effective in the short-term management of bleeding in kidney failure patients. The

effects of conjugated estrogen, used for long-term hemostasis, usually last for up to two weeks, compared with a few hours for DDAVP. Tranexamic acid, an antifibrinolytic agent administered in the form of a mouthrinse or soaked gauze significantly lowers operative and postoperative bleeding (Glick and Feagans, 2015).

Elective dental procedures should be carried out on the day after dialysis, when circulating toxins have been removed, the intravascular volume is high, and the products of heparin metabolism are at an ideal state (Proctor *et al*., 2005). At this time, the patient is able to tolerate dental treatment better. The anticoagulant effect of heparin used during dialysis does not cause residual bleeding abnormalities because its effect ends 3–4 hours after infusion (Lockhart *et al*., 2003).

As a result of alterations in fluid volume, sodium retention, and the presence of vascular access, these patients are commonly affected by a host of cardiovascular conditions. Often, hypertension, post dialysis hypotension, pulmonary hypertension and congestive heart failure can be seen in patients who are on hemodialysis. Hypertension in ESRD can lead to accelerated atherosclerosis. Although the medical management of these patients includes the aggressive use of antihypertensive drugs, the dentist should monitoring blood pressure at every visit, before and during dental procedures (Glick and Feagans, 2015). Avoiding excessive stress in the dental chair is important to minimize intraoperative systolic pressure elevations. The usage of sedative premedication should be considered for patients who are to undergo stressful procedures (Newadkar and Chaudhari, 2017).

Drug therapy are an important concern for dentists treating patients who have kidney disease. Most of drugs are excreted partially by the kidney, and kidney function affects drug bioavailability, volume of drug distribution, drug metabolism, and rate of drug elimination (Gupta and

Gupta, 2015). Certain medications are themselves nephrotoxic and should be avoided. Particular medications may be metabolized to acid and nitrogenous waste or may induce tissue catabolism. Non-steroidal anti-inflammatory drugs (NSAIDs) may induce sodium retention, prevent aldosterone production, impair the action of diuretics, affect renal artery perfusion, and cause acidosis. Tetracyclines and steroids are antianabolic, increasing urea nitrogen to approximately twice the baseline levels. Other drugs, such as phenacetin, are nephrotoxic and put added strain on an already deterioated kidney. The challenge for dental practitioners in prescribing medications is to preserve a therapeutic regimen within a narrow range, avoiding subtherapeutic dosing and toxicity (Glick and Feagans, 2015).

No studies have been reported on the use of topical fluoride in patients with kidney disease or on any related problems. If a patient with kidney disease needs fluoride supplements for caries control (particularly because of decreased salivary flow), the preferred way should be fluoride rinses until more definitive studies are performed (Glick and Feagans, 2015).

It is important to examine patients needing renal transplantation by a skilled dentist before surgery to determine which teeth can be maintained without representing an infection focus after renal transplant because of Infection is the major complication for renal transplant patients, that means a periodontal abscess, for example, is a potentially life-threatening condition (Hamid *et al*., 2006).

1.4. Saliva as a diagnostic fluid

Saliva is a unique biologic dynamic fluid that has varying spectrum of proteins, nucleic acids, polypeptides, electrolytes and hormones. It is an exocrine secretion of the salivary glands which is hypotonic in nature with a pH of 7.2–7.4 (Chicharro *et al*., 1998).The

diagnostic ability of saliva is reflected by the presence of multiple biomarkers which manifests at a concentration much lower than blood and still serves as a mirror reflecting the body's health and wellbeing (Malamud, 2011).

Saliva is a product of three major salivary glands: Parotid, submandibular, and sublingual with additional secretions from minor salivary gland (Batsakis, 1980). The variety of enzymes, antibodies, hormones, antimicrobial constituents, and growth factors are integrated into the saliva from the blood through the transcellular and paracellular routes (Rehak *et al*., 2000).

Saliva is earning importance in recent years and is considered a diagnostic tool for many reasons. Saliva is recognized to be functionally equivalent to the serum reflecting the physiological state of the body including hormonal, nutritional, emotional and metabolic variations (Lee and Wong, 2009). It is one of the easiest routes of collection of body fluid and is a simple noninvasive chair side procedure which does not need specialized equipment. Further it is associated with little complaints and is a cost-effective approach which is the most commonly used diagnostic tool for mass screening. It facilitates voluminous and repeated sampling in short periods of time. Considering these, saliva is identified as a potential diagnostic tool (Streckfus and Bigler, 2002; Malamud, 2011).

Parameters in saliva can be affected by numerous factors including diet and genetics. Because of this, use of saliva as a diagnostic fluid is still question to continuous research (Lasisi *et al*., 2016).

Many systemic diseases have been reported to produce remarkable and identifiable changes in salivary secretion. CKD is one of the systemic diseases that can affect the salivary secretion content. More importantly, saliva can indicate creatinine and urea levels in CKD patients which are the parameters commonly assessed in blood samples. Analysis of salivary

creatinine and urea in CKD patients offers numerous advantages that have been attributed to the use of saliva as a diagnostic fluid (Lasisi *et al*., 2016). Tomas *et al*. (2008) found higher salivary PH and higher salivary urea, sodium and potassium concentrations in patients with CKD than in healthy controls.

Utilization of blood for diagnostic tests is an invasive procedure generally associated with nervousness and distress to the patients. Also, some form of blood loss is usually related to procedures such as hemodialysis and frequent blood sampling in CKD patients. In addition, persons involved in the management of CKD patients are at more risk of blood borne diseases. Hence, a noninvasive diagnostic test with minimal risk with ability to provide a reliable evaluation of disease condition would be of worth to both the health professionals and the patients (Lasisi *et al*., 2016).

1.5. Kidney function markers

Creatinine, urea, uric acid and electrolytes are markers for routine analysis of kidney function whereas many studies have confirmed and consolidated the usefulness of markers such as cystatin C, β-Trace Protein (Gowda *et al*., 2010).

1.5.1. Creatinine

Creatinine is a 113-daltons waste product of muscle breakdown which is derived from phosphocreatine metabolism in muscle and also from meat ingestion or creatine supplement.

The influence of muscle mass on creatinine production affected by gender, age, race and body size (Stevens and Levey, 2005).

Creatinine excreted by the kidneys, therefore abnormal kidney function lead to increase serum creatinine level and less creatinine clearance through urine (Israni and Kasiske, 2011). The normal value of

serum creatinine is 0.6-1.5 mg/dl and salivary creatinine is 0.05-0.2 mg/dl (Venkatapathy *et al*., 2014).

Many studies observed a high creatinine level in serum and saliva of CKD patients (Davidovich *et al*., 2009; Xia *et al*., 2012; Venkatapathy *et al*., 2014). This is because inability of kidney to excrete creatinine and therefore increase its level in bloodstream (Guyton and Hall, 2006).

The increased salivary level of creatinine may be due to increased serum creatinine that creates a concentration gradient which facilitate the diffusion of creatinine from serum into saliva in patients with CKD (Nakahari *et al*., 1996).

1.5.2. Urea

Urea is the main nitrogenous end product of protein and amino acid catabolism, produced by liver and distributed throughout extracellular and intracellular fluid. In kidneys, urea is filtered out of blood by glomeruli and is partially being reabsorbed with water (Corbett, 2008). The most frequently determined clinical indicators for estimating kidney function depends upon urea concentration in the serum. It is helpful in differential diagnosis of acute renal failure and pre renal condition where blood urea nitrogen–creatinine ratio is increased (Mitchell and Kline, 2006).

Urea clearance is a poor index of glomerular filtration rate as its overproduction rate depends on many non-renal factors, involving diet and urea cycle enzymes. Increased blood urea nitrogen (BUN) is seen associated with renal disease or failure, congestive heart failure, blockage of the urinary tract by a kidney stone, fever, dehydration, Shock and bleeding in the digestive tract. The high BUN levels sometimes occur during late pregnancy or result from eating large amounts of protein-rich foods. If the BUN level is higher than 100 mg/dl it points to severe renal damage whereas decreased BUN level is observed in fluid excess. Low

levels are also seen in surgery, trauma, malnutrition, opioids and anabolic steroid use (Deska Pagana and Pagana, 2002).

Whenever there is a rise in the blood urea, there will be a concomitant rise in the salivary urea. Increase in blood urea concentration could lead to diffusion of nitrogenous waste products into other body fluids like gastric secretions, sweats and saliva. Increased salivary urea levels may indicate the need for treatment (Seethalakshmi *et al*., 2014). Normal blood urea concentration is 30-40 mg/dl whereas the normal salivary urea is 12-70 mg/dl (Kaushik *et al*., 2013).

1.6. Immunoglobulin A and chronic kidney disease:

Immunoglobulin A (IgA) is a serum immunoglobulin and the predominant antibody class in the external secretions that bathe mucosal surfaces, plays important roles in immune protection. In fact, the body expends great energy in producing IgA, such that the daily production of IgA exceeds that of all the other antibody classes gathered. This production rate suggests that, at least from an evolutionary standpoint, the benefits provided by IgA in terms of immune protection must be significant, in order to outweigh such energy costs to the body (Woof and Kerr, 2006).

Immunoglobulin A, at concentrations of about 2–3 mg/ml, is the second most common antibody in serum after IgG, which is normally present at about 12 mg/ml. Since serum IgA is metabolized some five times faster than IgG, the rates of production of serum IgA and IgG must be similar. While serum IgA is predominantly monomeric in nature, the IgA in secretions (secretory IgA, S-IgA) is chiefly polymeric, comprising mainly dimeric forms. S-IgA serves a set of functions to protect the vast surface area (approximately 400 m^2) occupied by mucosal surfaces, such as the linings of the gastrointestinal, respiratory and genitourinary tracts (Woof and Kerr, 2006).

IgA nephropathy is a glomerular disease characterized by deposition of IgA in the glomerular mesangium of the kidney, which was first reported by Berger in 1968 (Berger, 1968). It is the most prevalent form of glomerulonephritis in the world, accounting for about 30–45 % of all primary glomerular diseases (Yu and Chiang, 2014). IgA nephropathy can occur in all age groups, but is mainly found in patients in their 10s–30s, and it is more common in males, with a male to female ratio of 2:1 (Galla, 1965; Barratt and Feehally, 2005).

The disease can be diagnosed through biopsy by visualization of IgA deposition in the glomerular mesangium using immunofluorescence microscopy (Barratt and Feehally, 2005; Yu and Chiang, 2014). While IgA nephropathy has an indolent course, about 30 % of patients will reach ESRD after 20 years especially in those who present with hypertension, heavy proteinuria or renal insufficiency (Galla, 1995; Floege and Feehally, 2000; Barratt and Feehally, 2005).

On the other hands, patients with chronic kidney failure have secondary hyperparathyroidism and elevated blood levels of parathyroid hormone (PTH). Since PTH inhibits proliferation of B cell, it is, theoretically, possible that the chronic exposure of the B cells in uremic patients to excess PTH may adversely affect their ability to produce immunoglobulins in response to antigenic stimulation. Such a potential effect of PTH would provide, at least, a partial explanation for the impaired humoral immunity in chronic kidney failure (Gaciong *et al*., 1991).

1.7. Inflammatory status in chronic kidney disease

Chronic inflammation is common in patients with CKD. Many studies have explained that chronic inflammation may contribute to the morbidity and mortality among dialysis patients. In fact, deterioration of kidney function in uremia increases risk to infection and different abnormalities of the immune system. In addition, the repeated dialysis in patients lead to leukocyte activation and consequently the production of cytokines (Tbahriti *et al*., 2013).

The mechanisms through which inflammation leads to deterioration of the kidney function have not been fully explained. Immune-inflammatory mediators are known to modulate endothelial function, adhesion and interstitial migration of circulating immune cells (monocytes, leukocytes or neutrophils), besides being able of activating resident fibroblasts. The chronic inflammation observed in CKD is associated with increased serum levels of acute-phase protein reactants, such as C-reactive protein (CRP), and a variety of immune-inflammatory mediators, such as cytokines, components of the complement system, prostaglandins and leukotrienes. Interleukin-1 (IL-1), interleukin-6 (IL-6) and tumor necrosis factor α (TNF-α) are among the proinflammatory cytokines that have been related to the pathophysiology of kidney disease. There is an evidence of immune activation at the early stages of CKD in the adult people. Increased C-reactive protein levels have been associated with all-cause mortality in CKD patients. An association between the levels of CRP and proinflammatory cytokines, particularly IL-6, has also been detected (Vianna *et al*., 2011).

In dialysis patients, particularly hemodialysis, the repeated acute phase activation causes chronic inflammation, which produces in the long-term variability of complications, such as cardiovascular disease and malnutrition (Tbahriti *et al*., 2013).

1.7.1. Interleukin-6

Interleukin-6 is a 22–27 kilodalton polypeptide secreted from activated monocytes, macrophages, adipocytes, fibroblasts, and endothelial cells in response to various stimuli, such as TNF-α, IL-1β, physical exercise, bacterial endotoxins and oxidative stress. It is notable that whereas most other cytokines function via autocrine/paracrine mechanisms, the main effects of IL-6 are a consequence of its concentration in the circulation and can take place at sites distinct and far from its origin (Stenvinkel *et al*., 2002).

Although, a number of proinflammatory such as TNF-α and IL-1 and anti-inflammatory such as IL-10 cytokines coordinate the inflammatory response, available data suggest that IL-6 and its soluble receptor (sIL-6R) are central regulators of the inflammatory process (Jones *et al*., 2001). The IL-6 system promotes inflammatory events through lymphocyte activation and proliferation, differentiation of B cells, recruitment of leukocyte and the induction of the acute-phase protein response in the liver (Papanicolaou *et al*., 1998).

Considerable epidemiological information has lately linked plasma IL-6 to cardiovascular morbidity and mortality in non-renal patient groups (Ridker *et al*., 2000). Also, in ESRD patients an elevated IL-6 level is a strong foreteller of poor outcome (Pecoits-Filho *et al*., 2002).

The potential causes of high plasma level of IL-6 in ESRD patients may be related to

(i) The loss of kidney function,

(ii) Uremia *per se* (and its sequelae, such as fluid overload, oxidative stress and susceptibility to infections)

(iii) Dialysis-related factors. Even before the initiation of dialysis, patients with decreased kidney function already show signs of

inflammation and the deterioration of kidney function has been associated with a significant increase in serum cytokine levels (Descamps-Latscha *et al*., 1995).

Bolton *et al*. (2001), in a multiple regression analysis, found that serum creatinine was the sole specific determinant of IL-6 levels in a group of pre-dialysis and dialysis patients. One explanation for these findings might be the renal clearance impairment or inactivation of IL-6. In fact, ESRD patients have lower urinary IL-6 receptor excretion than controls (Memoli *et al*., 2000).

Both hemodialysis and peritoneal dialysis result in increased blood mononuclear cell IL-6 mRNA expression and plasma IL-6 levels (Takahashi *et al*., 2000). Many factors associated with hemodialysis have been suggested as contributing to the generation of IL-6 and/or enhancing the inflammatory effect of IL-6; dialysis against bioincompatible membrane, the use of non-sterile dialysate and backfiltration (Panichi *et al*., 1998; Memoli *et al*., 2000; Schiffl *et al*., 2001).

Caglar *et al*. (2002) showed that while the increase in IL-6 concentration was mild during the hemodialysis session, levels further increased at the end of the 2 hour post-hemodialysis period, providing clear evidence of a hemodialysis induced delayed inflammatory response.

Fluid overload and congestive heart failure may also contribute to increase IL-6 as kidney function decline. The circulating levels of IL-6 are elevated in chronic heart failure patients and both local and systemic effects of pro-inflammatory cytokines may be included in the pathogenesis of heart failure (Wollert and Drexler, 2001). Increased levels of IL-6 and C-reactive protein occur in patients with decompensated congestive heart failure (Sato *et al*., 1999).

1.7.2. C-reactive protein

C-reactive protein was first reported by Tillett and Francis in 1930 and was named so because it was found as a substance in the serum of patients with acute inflammation that reacted with the C-(capsular) polysaccharide of *Pnuemococcus* (Tillett and Francis, 1930).

C-reactive protein is an acute phase reactant that belongs to the protein family named as pentraxin. It is synthesized by the liver in response to cytokines such as interleukin-1, interleukin-6, TNF-α released from macrophages and adipocytes (Adejumo *et al*., 2016). CRP is the best characterized biomarker of inflammation (Devaraj *et al*., 2010) and used to determine the inflammatory status in several diseases (Rao et al., 2010). Chronic periodontitis may add to the inflammatory status of individuals and raise CRP levels (Gomes-Filho *et al*., 2011). Normal CRP levels vary among populations, with mean values between 2.5 and 5.0 mg/l (Correia and Burini, 2000).

Chronic kidney disease is a chronic inflammatory state caused by both patient and dialysis-related factors. These factors include the uremic milieu, oxidative stress, infection, obesity, genetic or immunologic factors, co-morbidities, exposure to dialyzer membrane and dialysate in those on dialysis (Jofre *et al*., 2009). Consequences of chronic inflammation in patients with CKD involve malnutrition, anemia, hypo-responsiveness to erythropoietin, cardiovascular disease and increased mortality (Pecoits-Filho *et al*., 2002; Menon *et al*., 2005; De Franciso *et al*., 2009).

C-reactive protein and serum albumin are markers of inflammation and independent risk factors for all-cause mortality in patients with CKD (Menon et al., 2005). The mortality of patients with ESRD remains high, with most deaths resulting from cardiovascular disease (Agadoa and Eggers, 1995). Many authors have found that overall mortality and

cardiovascular mortality were significantly higher in hemodialysis patients with increased CRP (Zimmermann *et al*., 1999; Yeun *et al*., 2000).

Annuk *et al*. (1992) reported that CRP and endothelial function could provide complementary prognostic information regarding future cardiovascular disorders in patients with kidney disease. Earlier reports have also established CRP as a valuable predictor of future cardiovascular events in CKD (Abraham *et al*., 2009; Jalal *et al*., 2012).

CHAPTER TWO

SUBJECT'S,

MARTIALS & METHODS

Subjects, Materials and Methods

2.1. Subjects:

A total of ninety (90) subjects were included in this study, divided into three groups:

A- Thirty patients with chronic kidney disease on hemodialysis for at least 6 months ago.

B- Thirty patients with chronic kidney disease on conservative treatment without hemodialysis.

C- Thirty healthy control participants with no signs and symptoms of any systemic disease.

The samples collection was done in Al-Kindey dialysis center at Al-Kindey teaching hospital in Baghdad during the period from December 2017 to the end of February 2018. Laboratory work was done by Al-Nadaer clinical laboratory.

After explaining the aims and objectives of the study, written informed consent was obtained from all participants (Appendix 1). Also, they were free and could withdraw from their participation at any time.

-Exclusion criteria:

1- Patients on chemotherapy or/and radiotherapy.
2- Patients on hemodialysis due to acute kidney failure or accident.
3- Hepatitis patients.

-Inclusion criteria:

1- Patients with CKD on conservative treatment.
2- Patients with CKD undergo hemodialysis (6 months and more).
3- Patients age 25-75 years.
4- Patients sign consent form.

2.1.1. Case sheet:

For all participants in this study, a case sheet was fulfilled and included the following information: (Appendix 2)

1- Patient demography (age, gender, occupation, marital state)
2- Risk factor such as smoking and alcohol consumption
3- Family history of chronic kidney disease
4- Medical history
5- History of present illness
6- Investigations (serum creatinine and blood urea)
7- Oral manifestations
8- Salivary parameters; salivary flow rate and PH

2.2. Materials:

The instruments, equipments and materials used in this study included the following:

- Disposable sterile gloves and masks.
- Disposable dental mirrors.
- Disposable sterile tubes for saliva collection.
- Timer.
- Digital PH meter.
- Ice box for saliva transport.
- Graduated plain tubes 10 ml for saliva centrifuge.
- Hettich EBA-20 Centrifuge.
- Eppendorf tubes 1.5 ml.
- Adjustable micropipette with disposable tips.
- Racks for eppendorf tubes.
- Freezer for saliva storage (- 20°C).
- Microplate reader Mindray MR-96A; Figure (2-1).
- Automated microplate washer – SAGA linear; Figure (2-2).

- JRAD 37°C Incubator.
- Roche - Cobas c 111 analyzer; Figure (2-3).
- Biolabo - Kenza max biochemistry analyzer; Figure (2-4).
- Cobas C111 - urea kit – Germany; Figure (2-5).
- Randox - creatinine kit – UK; Figure (2-6).
- Demeditec - secretory IgA ELISA kit – Germany; Figure (2-7)
- Shanghia biological - Human interleukin-6 ELISA kit- China; Figure (2-8).
- Shanghia biological - Human C-reactive protein ELISA kit- China; Figure (2-9).
- Disposable tubes used in procedures of parameters measurement.
- Absorbent paper.
- Distilled water.

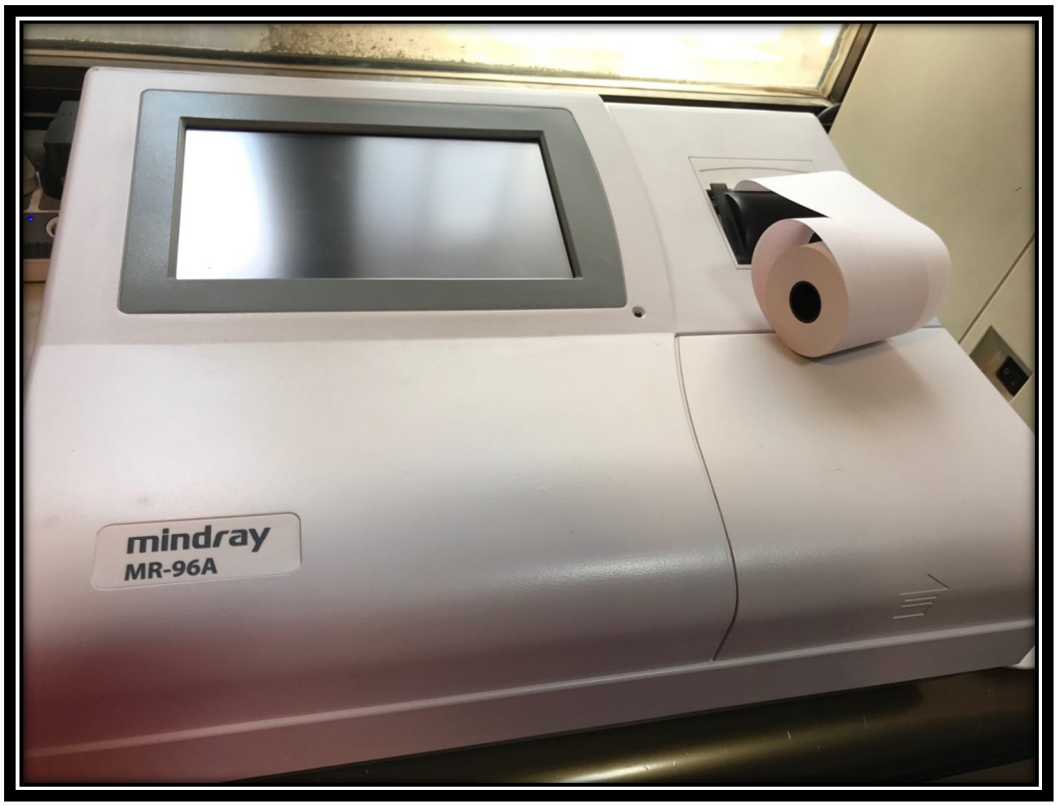


Figure (2-1): Microplate reader (Mindray MR-96A)

Figure (2-2): Automated microplate washer – SAGA linear

Figure (2-3): Roche - Cobas c 111 analyzer

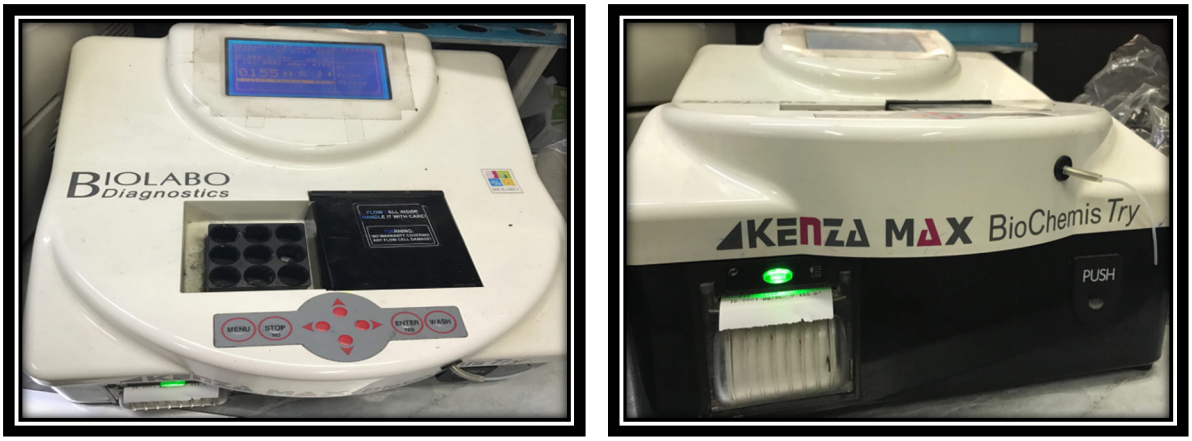


Figure (2-4): Biolabo - Kenza max biochemistry analyzer

Figure (2-5): Cobas C 111 – urea kit

Figure (2-6): Randox – creatinine kit

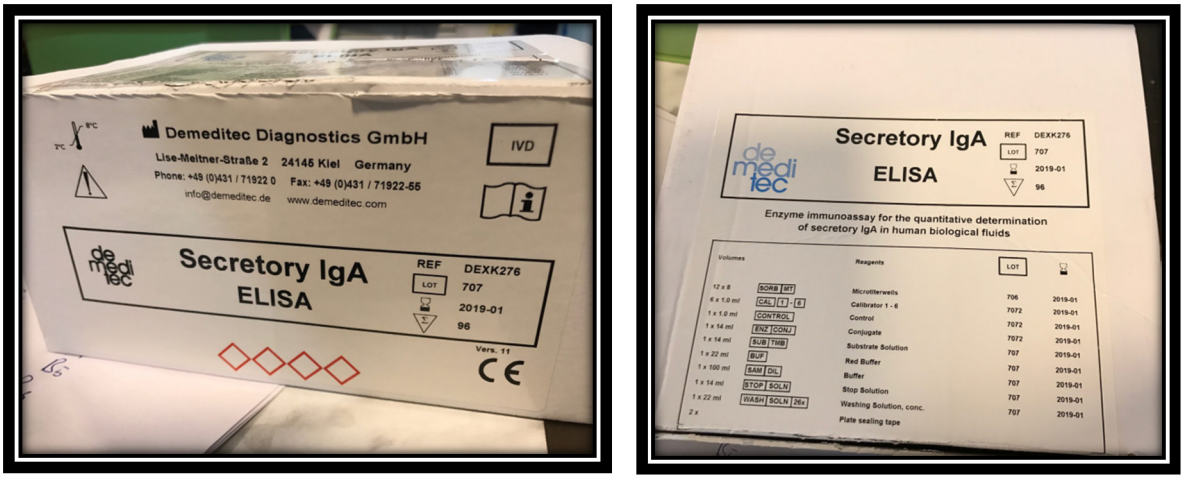
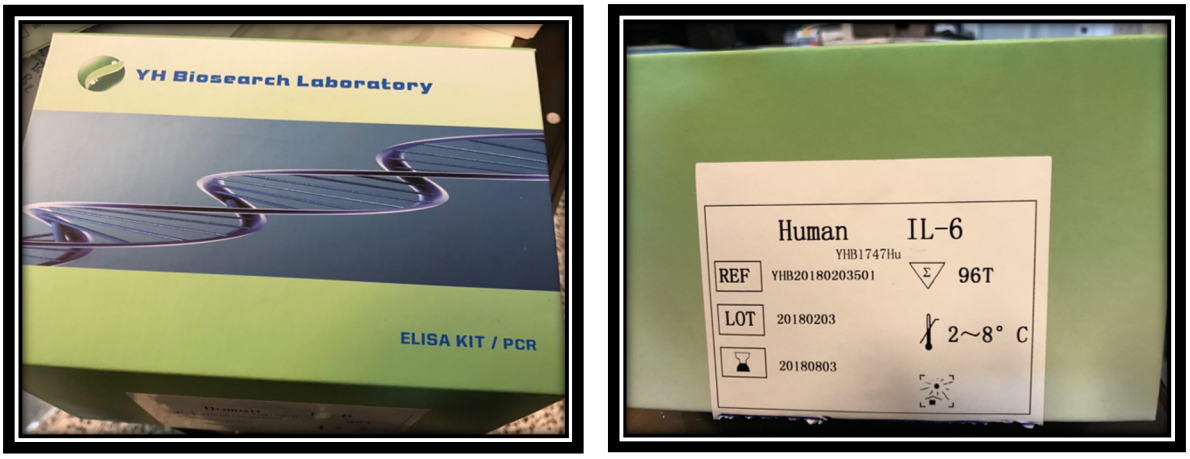


Figure (2-7): Secretory IgA ELISA kit



Figure(2-8): Human interleukin-6 ELISA kit

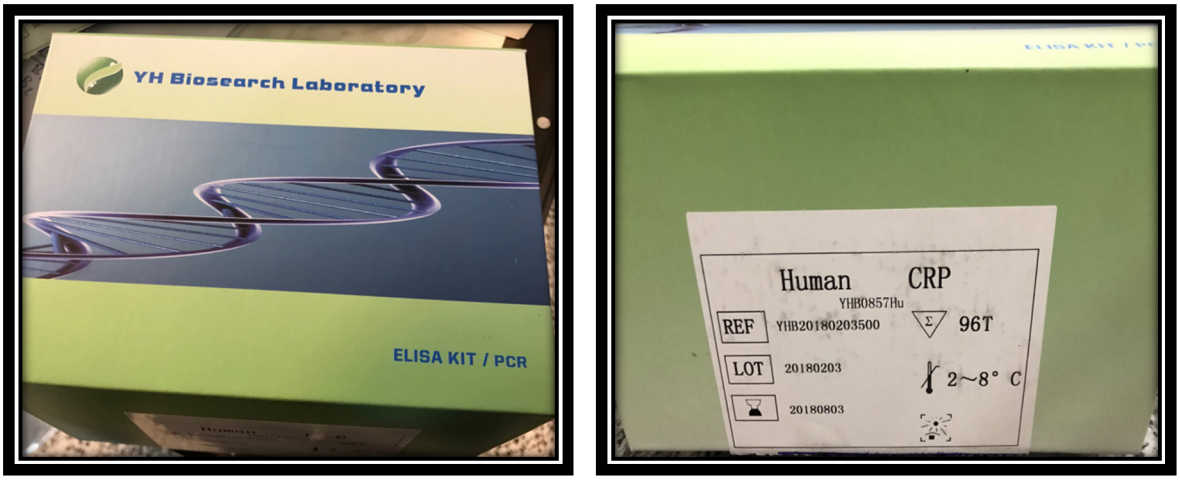


Figure (2-9): Human C- reactive protein ELISA kit

2.3. Methods:

2.3.1. Oral examination:

Oral examination was done for each participant using dental mirror with an artificial light with the oral manifestations were recorded in the case sheet (Appendix 1).

Oral findings that may be present in patients with CKD include the following:

- Uremic fetor (bad odor)
- Taste change
- Dry mouth
- Burning sensation
- Pale oral mucosa
- Mucosal petechiae and ecchymosis
- Uremic stomatitis
- Candidiasis
- Aphthus ulceration
- Angular cheilitis
- Gingival enlargement
- Enamel hypoplasia

2.3.2. Saliva collection:

After oral examination, saliva was collected from all individuals under the same conditions and each participant was instructed to rinse and wash his/her mouth with distilled water before saliva collection. Saliva was collected before meal or at least one hour after meal by spitting method for about 5-minutes. Sampling sessions limited to the hours between 9:00 and 11:00 AM to minimize the effect of diurnal variations.

The samples was identified by a code number during the time of sample collection and processing. After disappearance of the salivary froth, the salivary flow rate was calculated milliliters per minutes and pH was measured by digital pH meter. After collection of saliva samples, they were placed in a small cooler box to stop growth of bacteria and then, centrifuged at 3000 rpm for 10 minutes (Lasisi *et al*., 2015). The clear supernatant was taken and stored at -20°C until the time of analysis.

2.3.3. Estimation of study markers:

2.3.3.1. Estimation of creatinine in saliva:

Creatinine was estimated in saliva samples using randox creatinine manual kit and biolabo - kenza max biochemistry analyzer.

• **Colorimetric method** (Bartels *et al*., 1972)

• **Test principle**

Creatinine in alkaline solution reacts with picric acid to form a colored complex. The amount of the complex formed is directly proportional to the creatinine concentration.

• **Test procedure**

Using fresh double distilled water (ddH_2O) performed a new gain calibration in cuvette mode. CREA was selected in the run test screen and carried out a water blank as instructed.

Pipette into a cuvette: Table (2-1).

Table (2-1): Analysis of creatinine

	Reagent blank S0	**Standard SI**	**sample**
ddH2O	50 µl	-	-
Standard	-	50 µl	-
Sample	-	-	**50 µl**
Working reagent	500 µl	500 µl	**500 µl**

Mixed, inserted into biolabo – kenza max biochemistry analyzer and pressed read.

• Calculation

A2 – A1= Δ A sample or Δ A standard

A1: absorbance after 30 seconds

A2: absorbance exactly after 2 minutes later

$$\frac{\Delta A\ sample}{\Delta A\ standard} \times standard\ concentration\ \left({}^{mg}/_{dl}\right) = {}^{mg}/_{dl}$$

• Reagents composition

- CAL. Standard
- Ria. Picric acid 35 mmol/l
- Rib. Sodium hydroxide 0.35 mol/l

2.3.3.2. Estimation of urea in saliva:

Salivary urea was measured by Roche – Cobas C 111 analyzer automatically by using cobas ureal kit.

• Test principle

Kinetic test with urease and glutamate dehydrogenase (Talke and Schubert, 1965; Tiffany *et al*., 1972; Richterich and Colombo, 1978; Sampson *et al*., 1980).

Urea is hydrolyzed by urease to form ammonium and carbonate.

$$\text{Urea} + 2\ H2O \xrightarrow{urease} 2\ NH4+ + CO3^{2}-$$

In the second reaction 2-oxoglutarate reacts with ammonium in the presence of glutamate dehydrogenase (GLDH) and the coenzyme NADH to produce L-glutamate. In this reaction two moles of NADH are oxidized to NAD for each mole of urea hydrolyzed.

$$NH4+ + \text{2-oxoglutarate} + NADH \xrightarrow{GLDH} \text{L-glutamate} + NAD+ + H2O$$

The rate of decrease in the NADH concentration is directly proportional to the urea concentration in the specimen and is measured photometrically.

• Calculation

The cobas c 111 analyzer automatically calculated the urea concentration of each sample.

Reagents – working solution •

R1 TRIS buffer: 220 mmol/L, PH 8.6; 2-oxoglutarate: 73 mmol/L; NADH: 2.5 mmol/L; ADP: 6.5 mmol/L; urease (jack bean): ≥ 300 µkat/L; GLDH (bovine liver): ≥ 80 µkat/L; preservative.

2.3.3.3. Estimation of secretory IgA in saliva:

Secretory IgA in saliva sample was measured by Microplate reader Mindray MR-96A using ELISA kit for IgA.

• Principle of the test

This test is based on two-site sandwich enzyme immunoassay principle. Tested specimen is placed into the microwells coated by specific murine monoclonal to human secretory IgA-antibodies. Antigen from the specimen is captured by the antibodies coated onto the microwell surface. Unbound material is removed by washing procedure.

Second antibodies – murine monoclonal to human IgA alfa chain, labelled with peroxidase enzyme, are then added into the microwells. After subsequent washing procedure, the remaining enzymatic activity bound to the microwell surface is detected and quantified by addition of chromogen-substrate mixture, stop solution and photometry at 450 nm. Optical density in the microwell is directly related to the quantity of the measured analyte in the specimen.

• **Assay procedure**

1-The desired number of microstrips were placed into the frame; allocate 14 wells for the calibrators CAL 1 – 6 and control samples control and two wells for each unknown sample. Adhesive sealing tape from unused strips should not be removed. The calibrator/control and unknown sample wells were filled differently.

2- Samples were diluted using buffer DIL SPE (EIA buffer) 101 fold. (5 µl of saliva sample+ 500 µl of diluent). Control sample and calibrators should not be diluted.

3-If suggested analyte concentration in the sample exceeds the highest calibrator, additionally this sample was diluted accordingly, using DIL SPE (EIA buffer). Use of other buffers or reagents for sample dilution may lead to incorrect measurement.

4-190 µl of red EIA buffer was pipetted into the wells allocated for saliva.

5-100 µl of calibrators CAL 1 – 6 and control samples (control) were pipetted into allocated wells. For testing of saliva 10 µl of the unknown sample was pipetted into the allocated wells. The contents of the wells was mixed carefully by short horizontal rotating of the plate for 5-7 seconds and the wells was covered by plate adhesive tape (included into the kit).

6-Incubation was done 90 minutes at 37 °C.

7- Washing solution was prepared by 21x dilution of washing solution concentrate BUF WASH 21X by distilled water. Minimal quantity of washing solution should be 250 µl per well. Strips were washed 3 times.

8-100 µl of CONJ HRP was dispensed into the wells. The wells were covered by plate adhesive tape.

9-Incubation was done 30 minutes at 37 °C.

10- The strips were washed 5 times.

11- 100 µl of SUBS TMB was dispensed into the wells.

12- Incubation was done 10-20 minutes at 18-25 °C.

13- 100 µl of STOP was dispensed into the wells.

14- Optical density (OD) was measured at 450 nm.

15- Photometer blank was set on first calibrator

16- Point-by-point method for data reduction was applied. Calculation factor (1.0) was used, the calculation and contents of the kit are mentioned in (Appendix 3).

2.3.3.4. Estimation of interleukin- 6 in saliva:

Interleukin-6 in saliva sample was measured by Microplate reader Mindray MR-96A using ELISA kit for interleukin-6.

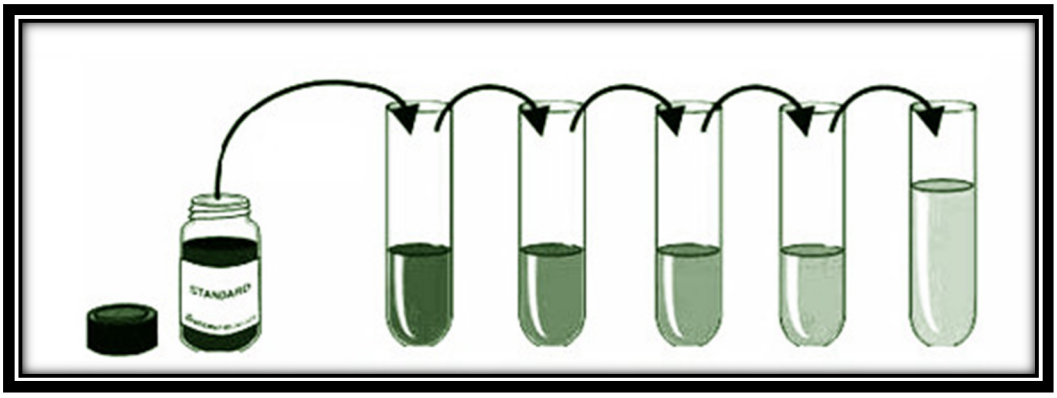
• Principle of the test

This kit used enzyme-linked immune sorbent assay (ELISA) based on biotin double antibody sandwich technology to assay Human Interleukin 6(IL-6). Interleukin 6(IL-6) was added to wells that are pre-coated with Interleukin 6(IL-6) monoclonal antibody and then incubated. After incubation, Anti IL-6 antibodies labeled with biotin was added to unite with streptavidin-HRP, which forms the immune complex. Unbound enzymes was removed after incubation and washing, then substrate A and B was added. The solution was turned to blue and changed to yellow with the effect of acid. The shades of solution and the concentration of Human Interleukin 6(IL-6) are positively correlated.

• **Assay procedure:**

a) Dilution of standard solutions: This kit provided one standard original concentration, so the dilution may independently be done in small tubes following the chart below:

320ng/L	**Standard No.5**	**120µl Original Standard + 120µl Standard diluents**
160ng/L	Standard No.4	120µl Standard No.5 + 120µl Standard diluents
80ng/L	Standard No.3	120µl Standard No.4 + 120µl Standard diluent
40ng/L	Standard No.2	120µl Standard No.3 + 120µl Standard diluent
20ng/L	Standard No.1	120µl Standard No.2 + 120µl Standard diluent



Standard solution No.5 No.4 No.3 No.2 No.1

b) The number of stripes needed was determined by that of samples to be tested added by the standards. It is recommended that each standard solution and each blank well be arranged with multiple wells as much as possible.

c) Sample injection: 1) Blank well: sample, anti IL-6 antibody labeled with biotin and streptavidin-HRP should not be added; chromogen reagent A & B and stop solution was added, each other step operation was the same. 2) Standard solution well: 50µl standard and streptomycin-HRP 50µl was added (biotin antibodies have united in advance in the standard so no biotin antibodies are added). 3) Sample well to be tested: 40µl sample was added and then 10µl IL-6 antibodies, 50µl streptavidin-

HRP. Then it was covered with seal plate membrane. It was shaken gently to mix. Incubation was done at 37℃ for 60 minutes.

d) Preparation of washing solution: The washing concentration was diluted (30X) with distilled water for later use.

e) Washing: The seal plate membrane was carefully removed, liquid was drained and the remainder was shake off. Each well was filled with washing solution, stayed for 30 seconds, then drained. This procedure was repeated five times then the plate was blotted.

f) Color development: First 50µl chromogen reagent A was added to each well, and then 50µl chromogen reagent B was added to each well. It was shaken gently to mix.

Incubation was done for 10 minutes at 37℃ away from light for color development.

g) Stop: 50µl Stop Solution was added to each well to stop the reaction (color changed from blue to yellow at that moment).

h) Assay: Blank well was taken as zero, the absorbance (OD) of each well was measured one by one under 450 nm wavelength, which should be conducted within 10 minutes after having added stop solution.

i) According to standards concentrations and corresponding OD values, the linear regression equation of the standard curve was calculated. Then according to the OD value of samples, the concentration of the corresponding sample was calculated. Statistical software could also be employed, the calculation and contents of the kit are mentioned in (Appendix 4).

2.3.3.5. Estimation of C-reactive protein in saliva:

C-reactive protein in saliva sample was measured by Microplate reader Mindray MR-96A using ELISA kit for CRP.

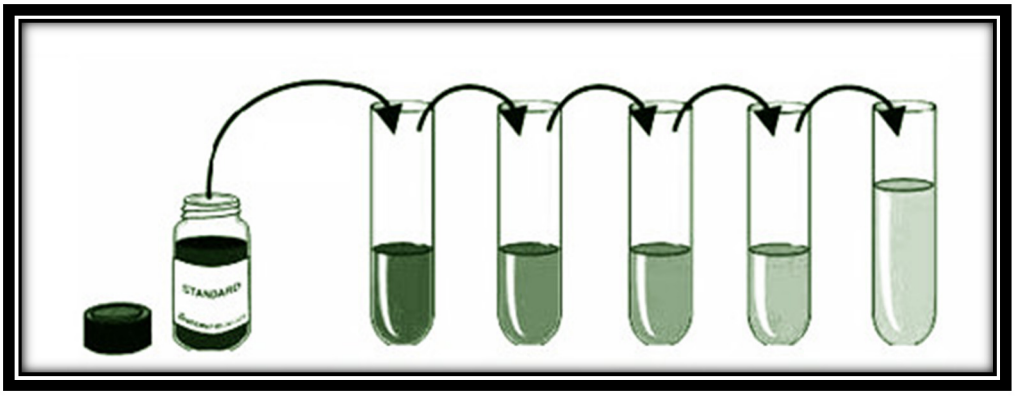
• **Principle of the test**

This kit used enzyme-linked immune sorbent assay (ELISA) based on biotin double antibody sandwich technology to assay Human C-Reactive Protein (CRP). C-Reactive Protein (CRP) was added to wells that are pre-coated with C-Reactive Protein (CRP) monoclonal antibody and then incubation was done. After incubation, anti CRP antibodies labeled with biotin was added to unite with streptavidin-HRP, which forms the immune complex. Unbound enzymes was removed after incubation and washing, then substrate A and B was added. The solution was turn to blue and changed to yellow with the effect of acid. The shades of solution and the concentration of Human C-Reactive Protein (CRP) are positively correlated.

• **Assay procedure**

a) Dilution of standard solutions: This kit provided one standard original concentration. So the dilution may independently be done in small tubes following the chart below:

3.6mg/L	**Standard No.5**	**120µl Original Standard + 120µl Standard diluents**
1.8mg/L	Standard No.4	120µl Standard No.5 + 120µl Standard diluents
0.9mg/L	Standard No.3	120µl Standard No.4 + 120µl Standard diluent
0.45mg/L	Standard No.2	120µl Standard No.3 + 120µl Standard diluent
0.225mg/L	Standard No.1	120µl Standard No.2 + 120µl Standard diluent



Standard solution No.5 No.4 No.3 No.2 No.1

b) The number of stripes needed was determined by that of samples to be tested added by the standards. It is recommended that each standard solution and each blank well be arranged with multiple wells as much as possible.

c) Sample injection: 1) Blank well: Sample, anti CRP antibody labeled with biotin and streptavidin-HRP should not be added; chromogen reagent A & B and stop solution was added, each other step operation was the same. 2) Standard solution well: 50µl standard and streptomycin-HRP 50µl was added (biotin antibodies have united in advance in the standard so no biotin antibodies are added). 3) Sample well to be tested: 40µl sample was added and then 10µl CRP antibodies, 50µl streptavidin-HRP. Then it was covered with seal plate membrane. It was shaken gently to mix. Incubation was done at 37℃ for 60 minutes.

d) Preparation of washing solution: The washing concentration (30X) was diluted with distilled water for later use.

e) Washing: The seal plate membrane was carefully removed, drain liquid and the remainder was shake off. Each well filled with washing solution, stayed for 30 seconds, then drained. This procedure was repeated five times then the plate was blotted.

f) Color development: First 50µl chromogen reagent A was added to each well, and then 50µl chromogen reagent B was added to each well. It was shaken gently to mix. Incubation was done for 10 minutes at 37℃ away from light for color development.

g) Stop: 50µl Stop Solution was added to each well to stop the reaction (color changed from blue to yellow at that moment).

h) Assay: Blank well was taken as zero, the absorbance (OD) of each well was measured one by one under 450 nm wavelength, which should be conducted within10 minutes after having added stop solution.

i) According to standards concentrations and corresponding OD values, calculate the linear regression equation of the standard curve was calculated. Then according to the OD value of samples, the concentration of the corresponding sample was calculated. Statistical software could also be employed, the calculation and contents of the kit are mentioned in (Appendix 5).

2.4. Statistical analysis:

Statistical analysis was performed with SPSS (Statistical Package for Social Sciences; Version 21). Descriptive statistical analysis, contingency coefficient, Fisher exact, Chi-Square, student T-test, Analysis of Variance (ANOVA), Post-hoc (LSD and Dunnett T3) tests and Person Correlation Coefficient (r) were used in this study.

$P < 0.05$ significant

$P < 0.01$ highly significant

$P > 0.05$ non-significant

CHAPTER THREE

RESULTS

Results

3.1. Clinical findings

3.1.1. Demographic findings

A total of ninety participants were included in this study. The age range at first presentation was 25-75 years with an overall mean age of 49.63 years (SD ± 12.23). 46 of them were males with a mean age of 47.89 (SD: 10.56) and 44 of them were females with a mean age of 51.45 (SD ± 13.64).

One third of them were retired (34.4%) followed by unemployed (26.67%), workers (24.4%) and officers (14.4%).

The majority of study population were married (74.4%) followed by singles (17.7%), widowed (5.5%) and divorced (2.2%).

3.1.1.1. Age and gender

-Thirty patients were with CKD on hemodialysis with an age range of 37-75 years and a mean age of 55.367 (SD ± 9.87). Seventeen of them were males and 13 were females.

-Thirty patients were with CKD with conservative treatment with an age range of 25-75 years and a mean age of 49.300 (SD ± 13.96). Sixteen of them were males and 14 were females.

-Thirty healthy subjects with an age range of 27-63 years and a mean age of 44.233 (SD ± 10.11). Thirteen of them were males and 17 were females.

The participants classified into two age categories: (≤ 50) and (more than 50 years of age).

A statistical analysis using contingency coefficient revealed that there was no significant difference among studied groups in relation to age (P= 0.06) or gender (P= 0.56); table (3-1).

Table (3-1): Distribution of CKD patients and control groups according to age and gender.

			Groups			C.C	P value
			CKD (HD)	CKD	Control		
Age	≤ 50	No. (%)	11 (36.6)	17 (56.6)	20 (66.6)	0.243	**0.060 NS**
	More than 50	No. (%)	19 (63.3)	13 (43.3)	10 (33.3)		
Gender	Male	No. (%)	17 (56.6)	16 (53.3)	13 (43.3)	0.113	**0.561 NS**
	Female	No. (%)	13 (43.3)	14 (46.6)	17 (56.6)		

C.C: contingency coefficient
CKD (HD): chronic kidney disease on hemodialysis
CKD: chronic kidney disease on conservative treatment

3.1.1.2. Occupation and marital state

Thirty one (34.4%) of the participants were retired followed by 24 (26.67%) were unemployed then 22 (24.4%) were workers and 13 (14.4%) were officers.

In CKD patients on hemodialysis, the majority were unemployed 13 (43.3%) followed by 9 (30%) were retired, 4 (13.3%) were workers and 4 (13.3%) were officers. In those on conservative treatment most of them were retired 14 (46.6%) followed by 10 (33.3%) were workers, 3 (10%) were unemployed and 3 (10%) were officers. While in control group, 8 (26.6%) were retired, 8 (26.6%) were unemployed, 8 (26.6%) were workers and 6 (20%) were officers.

A statistical analysis using contingency coefficient showed that there was no significant difference among studied groups regarding occupation (p= 0.06), table (3-2).

Table (3-2): Distribution of occupation among studied groups.

			Groups			C.C	P value
			CKD (HD)	CKD	Control		
Occupation	Retired	No. (%)	9 (30)	14 (46.6)	8 (26.6)	0.34	**0.065 NS**
	Unemployed	No. (%)	13 (43.3)	3 (10)	8 (26.6)		
	Worker	No. (%)	4 (13.3)	10 (33.3)	8 (26.6)		
	Officer	No. (%)	4 (13.3)	3 (10)	6 (20)		

C.C: contingency coefficient
CKD (HD): chronic kidney disease on hemodialysis
CKD: chronic kidney disease on conservative treatment

Considering marital status, married participants were the most common 67 (74.4%) followed by single 16 (17.7%), widowed 5 (5.5%) and divorced 2 (2.2%).

In CKD patients on hemodialysis, the majority were married 27 (90%) and 3 (10%) were single. In those on conservative treatment, 19 (63.3%) were married followed by 6 (20%) were single, 3 (10%) were widowed and 2(6.6%) were divorced. While in control group, 21 (70) were married followed 7 (23.3%) were single and 2 (6.6%) were widowed.

Also, there was no significant difference among studied groups considering marital status (p= 0.21); table (3-3).

Table (3-3): Marital status of CKD patients and control group.

			Groups			C.C	P value
			CKD (HD)	CKD	Control		
Marital status	Married	No. (%)	27 (90)	19 (63.3)	21 (70)	0.31	**0.21 NS**
	Single	No. (%)	3 (10)	6 (20)	7 (23.3)		
	Widowed	No. (%)	0 (00)	3 (10)	2 (6.6)		
	Divorced	No. (%)	0 (00)	2 (6.6)	0 (00)		

C.C: contingency coefficient
CKD (HD): chronic kidney disease on hemodialysis
CKD: chronic kidney disease on conservative treatment

3.1.2. Salivary flow rate and PH

3.1.2.1. Salivary flow rate

The present study showed that there was a significant salivary flow rate difference among studied groups by using ANOVA test as shown in table (3-4).

Table (3-4): Mean salivary flow rate of studied groups with ANOVA test.

	Groups	Mean ± SD	SE	Range	F	P value
Salivary flow rate (ml/min)	CKD (HD)	0.52 ± 0.30	0.05	0.20-1.40	7.293	**0.001 S**
	CKD	0.40 ± 0.14	0.02	0.20-0.70		
	Control	0.63 ± 0.26	0.05	0.30-1.60		

F: ANOVA
CKD (HD): chronic kidney disease on hemodialysis
CKD: chronic kidney disease on conservative treatment

Then by using post hoc (dunnett T3) test, the result showed that there was a significant decrease in salivary flow rate of CKD patients

with conservative treatment compared to the control group (p= 0.00).

Although, no significant difference in salivary flow rate between CKD on hemodialysis and control group was found (p= 0.30), the mean salivary flow rate in hemodialysis patients was lower compared to control group. Also, no significant difference in salivary flow rate between CKD on hemodialysis and those on conservative treatment (p= 0.13) as shown in table (3-5).

Table (3-5): Post hoc (dunnett T3) test of salivary flow rate among studied group.

		Group (I)	Group (J)	MD	P value
Salivary flow rate (ml/min)	Dunnett T3	CKD (HD)	Control	-0.12	**0.30 NS**
			CKD	0.12	**0.13 NS**
		Control	CKD	0.24	**0.00 HS**

MD: mean difference
CKD (HD): chronic kidney disease on hemodialysis
CKD: chronic kidney disease on conservative treatment

3.1.2.2. Salivary PH

Table (3-6) shows that there was a significant difference among studied groups in relation to salivary PH by using ANOVA test.

Table (3-6): Mean salivary PH of studied groups with ANOVA test.

	Groups	Mean ± SD	SE	Range	F	P value
Salivary PH	CKD (HD)	7.95 ± 0.51	0.09	6.60-8.80	11.247	**0.000**
	CKD	7.62 ± 0.50	0.09	6.70-8.50		
	Control	7.34 ± 0.49	0.09	6.20-8.20		**HS**

F: ANOVA
CKD (HD): chronic kidney disease on hemodialysis
CKD: chronic kidney disease on conservative treatment

Then using post hoc (LSD) test, the result revealed that there was a significant higher salivary PH in CKD patients on hemodialysis and those on conservative treatment compared to control group (p= 0.00, p= 0.03), While there was no significant salivary PH difference between CKD patients on hemodialysis and those on conservative treatment (p= 0.051) as shown in table (3-7).

Table (3-7): Post hoc (LSD) test of salivary PH among studied groups.

		Group (I)	Group (J)	MD	P value
Salivary PH	LSD	CKD (HD)	Control	0.61	**0.00 HS**
			CKD	0.33	**0.051 NS**
		Control	CKD	-0.28	**0.03 S**

MD: mean difference
CKD (HD): chronic kidney disease on hemodialysis
CKD: chronic kidney disease on conservative treatment

3.1.3. Smoking status

The majority of the participants were non-smoker 71 (78.89%) followed by current smoker 14 (15.56%) and ex-smoker 5 (5.56%).

In CKD patients on hemodialysis, 26 (86.6%) were non-smokers, 3 (10%) were ex-smokers and 1 (3.3%) were current smokers. In those on conservative treatment, 22 (73.3%) were non-smokers, 2 (6.6%) were ex-smokers and 6 (20%) were current smokers. While in control group, 23 (76.6%) were non-smokers and 7 (23.3) were current smokers.

By using Fisher exact test, the result showed that there was no significant difference among studied groups in relation to smoking status (p= 0.058); table (3-8).

Table (3-8): Smoking status in CKD patients and control group.

Smoking status		Groups			Fisher exact	P value
		CKD (HD)	CKD	Control	8.07	**0.058 NS**
Nonsmoker	No. (%)	26 (86.6)	22 (73.3)	23 (76.6)		
ex-Smoker	No. (%)	3 (10)	2 (6.6)	0 (00)		
Current smoker	No. (%)	1 (3.3)	6 (20)	7 (23.3)		

CKD (HD): chronic kidney disease on hemodialysis
CKD: chronic kidney disease on conservative treatment

Current smokers were divided into three groups according to the number of cigarettes smoked per day: light (< 10), intermediate (10-20), and heavy smokers (> 20). Overall, intermediate smokers were the most common (11).

In CKD patients on hemodialysis, 1 (7.1%) was intermediate smoker. in those on conservative treatment, 5 (35.7%) were intermediate smokers and 1 (7.1%) was heavy smoker. While in control group, 1 (7.1%) was light smoker, 5 (35.7%) were intermediate smokers and 1 (7.1%) was heavy smoker.

Also, by using Fisher exact test, the result showed that there was no significant difference among studied groups regarding to numbers of cigarettes (p= 1.00); table (3-9).

Table (3-9): Current smoker categories according to the numbers of cigarette.

No. of cigarettes per day		Groups			Fisher exact	P value
		CKD (HD)	CKD	Control		
1-10	No. (%)	0 (00)	0 (00)	1 (7.1)	3.03	**1.00 NS**
10-20	No. (%)	1 (7.1)	5 (35.7)	5 (35.7)		
21+	No. (%)	0 (00)	1 (7.1)	1 (7.1)		

CKD (HD): chronic kidney disease on hemodialysis
CKD: chronic kidney disease on conservative treatment

3.1.4. Medical history

In relation to patients' medical history, 35 (58.33%) of CKD patients were with diabetes mellitus, 34 (56.67%) were with hypertension and 21 (35%) were with diabetes and hypertension.

In patients on hemodialysis, 14 (46.67%) were diabetic, 19 (63.33%) were hypertensive and 11 (36.6%) were with diabetes and hypertension. While in those on conservative treatment, 21 (70%) were diabetic, 15 (50%) were hypertensive and 10 (33.3%) were with diabetes and hypertension.

By using Chi-square test, the result showed that there was no significant difference between patients groups regarding diabetic and hypertension (p= 0.06, p= 0.29, p= 0.78); table (3-10).

Table (3-10): Medical history of chronic kidney disease patients.

		Groups		Chi-square	P value
		CKD (HD)	CKD		
Diabetes	No. (%)	14 (46.6)	21 (70)	3.36	**0.067 NS**
Hypertension	No. (%)	19 (63.3)	15 (50)	1.08	**0.29 NS**
Diabetes and hypertension	No. (%)	11 (36.6)	10 (33.3)	0.07	**0.78 NS**

CKD (HD): chronic kidney disease on hemodialysis
CKD: chronic kidney disease on conservative treatment

3.1.5. Family history

Considering family history, 12 (20%) CKD patients were with 1st relative degree history of CKD and 2 (3.3%) were with 2nd relative degree.

In hemodialysis patients, 5 (16.6%) were with 1st relative degree and 1 (3.3%) was with 2nd relative degree family history of CKD. While in patients on conservative treatment, 7 (23.3%) were with 1st relative degree and 1 (3.3%) were with 2nd relative degree family history.

Using Chi-square test, the result revealed that there was no significant difference between patients groups in relation to family history (p= 0.51, p= 1.00); table (3-11).

Table (3-11): Family history of chronic kidney disease patients.

		Groups		Chi-square	P value
		CKD (HD)	CKD		
1st degree	No. (%)	5 (16.6)	7 (23.3)	0.41	**0.51 NS**
2nd degree	No. (%)	1 (3.3)	1 (3.3)	0.00	**1.00 NS**

CKD (HD): chronic kidney disease on hemodialysis
CKD: chronic kidney disease on conservative treatment

3.2. Oral findings

In CKD on hemodialysis, uremic fetor was seen in 15 (50%) followed by dry mouth in 14 (46.6%), taste change in 13 (43.3%), Pale oral mucosa in 12 (40%), aphthus ulceration in 7 (23.3%), gingival enlargement in 4 (13.3%), burning sensation in 2 (6.6%) and angular cheilitis in 1 (3.3%) of patients; Figure (3-1).

In those on conservative treatment, taste change was seen in 14 (46.6%) followed by dry mouth in 11 (36.6%), uremic fetor in 10 (33.3%), Pale oral mucosa in 6 (20%), aphthus ulceration in 5 (16.6%),

gingival enlargement in 5 (16.6%), burning sensation in 3 (10%) and angular cheilitis in 1 (3.3%) of patients; Figure (3-2).

No significant differences were found in oral findings between CKD patients on hemodialysis and CKD patients with conservative treatment; tables (3-12).

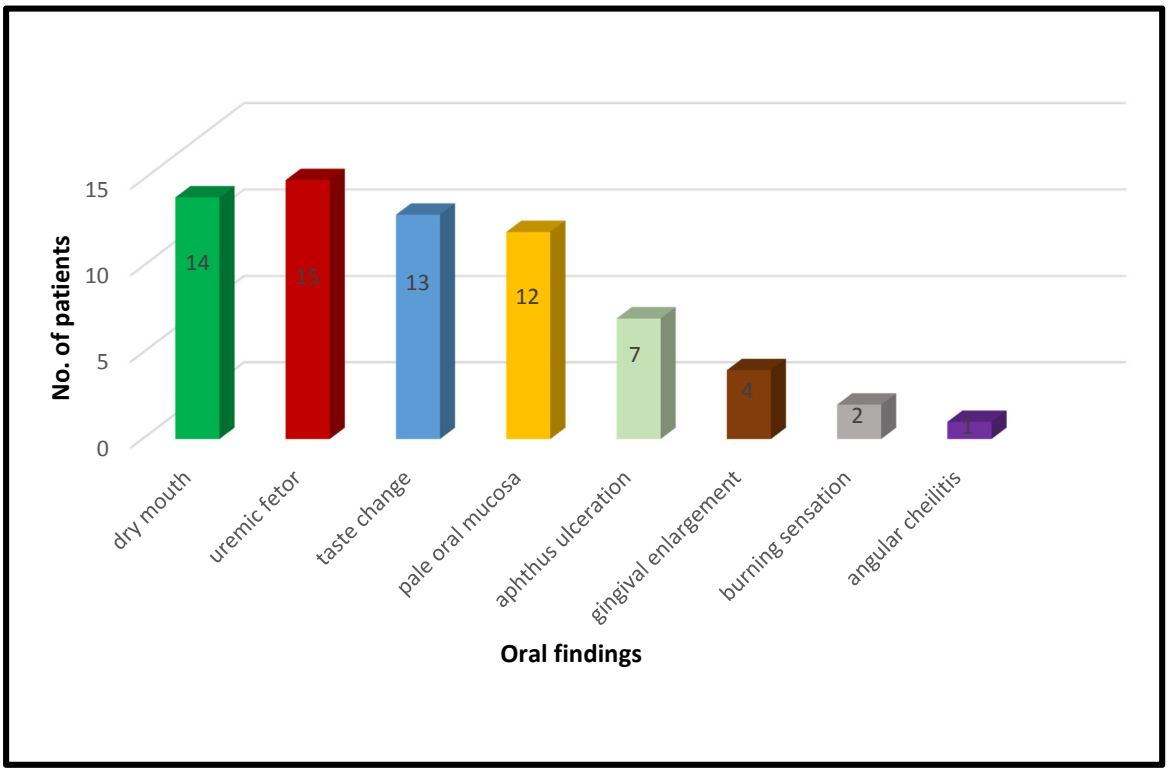


Figure (3-1): Oral findings in CKD patients on hemodialysis.

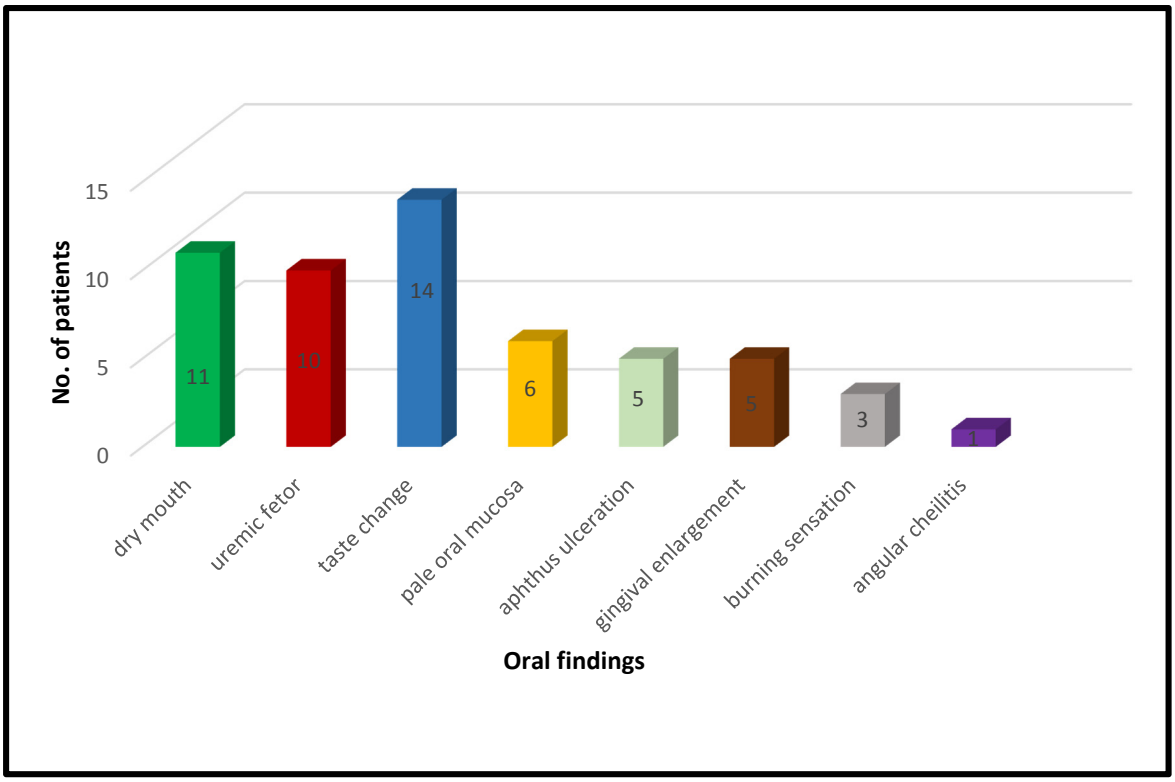


Figure (3-2): Oral findings in CKD patients on conservative treatment.

Table (3-12): Oral findings in CKD patients on hemodialysis and on conservative treatment with contingency coefficient.

		Group		C.C	P value
		CKD (HD)	CKD		
Dry mouth	No. (%)	14 (46.6)	11 (36.6)	0.101	**0.432 NS**
Uremic fetor	No. (%)	15 (50)	10 (33.3)	0.16	**0.190 NS**
Taste change	No. (%)	13 (43.3)	14 (46.6)	0.03	**0.795 NS**
Pale oral mucosa	No. (%)	12 (40)	6 (20)	0.21	**0.091 NS**
Aphthus ulceration	No. (%)	7 (23.3)	5 (16.6)	0.08	**0.519 NS**
Gingival enlargement	No. (%)	4 (13.3)	5 (16.6)	0.04	**0.718 NS**
Burning sensation	No. (%)	2 (6.6)	3 (10)	0.060	**0.640 NS**
Angular cheilitis	No. (%)	1 (3.3)	1 (3.3)	0.00	**1.00 NS**

C.C: contingency coefficient
CKD (HD): chronic kidney disease on hemodialysis
CKD: chronic kidney disease on conservative treatment

3.3. Laboratory findings

3.3.1. Salivary and serum creatinine

Figure (3-3) shows the mean serum and salivary creatinine in CKD patients on hemodialysis and on conservative treatment with salivary creatinine in control group.

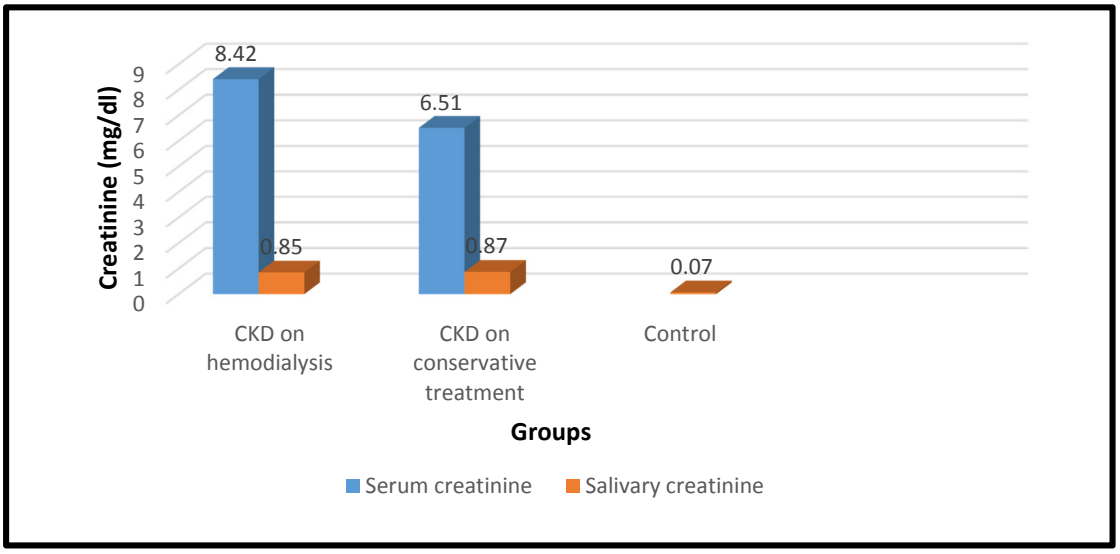


Figure (3-3): Mean serum and salivary creatinine in CKD patients and control group.

There was a significant difference in salivary creatinine of CKD patients on hemodialysis, those on conservative treatment and control group using ANOVA test; table (3-13).

Table (3-13): Mean salivary creatinine of studied groups with ANOVA test.

	Groups	mean± SD	SE	Range	F	P value
Salivary creatinine (mg/dl)	CKD(HD)	0.85 ± 0.38	0.07	0.36-1.99	11.44	**0.00**
	CKD	0.87 ± 1.22	0.22	0..30- 7.24		
	Control	0.07 ± 0.08	0.01	0.01 - 0.036		**S**

F: ANOVA
CKD (HD): chronic kidney disease on hemodialysis
CKD: chronic kidney disease on conservative treatment

Using post hoc (LSD) test, there was a significant increase in salivary creatinine level in both CKD patients on hemodialysis and those on conservative treatment compared to control group (p= 0.00, p= 0.00). While, no significant salivary creatinine difference was found between patients on hemodialysis and those with conservative treatment (p= 0.90); table (3-14).

Table (3-14): Post hoc (LSD test) of salivary creatinine among studied groups.

		Group (I)	Group (J)	MD	P value
Salivary creatinine (mg/dl)	LSD	CKD (HD)	Control	0.78	**0.00 HS**
			CKD	-0.02	**0.90 NS**
		Control	CKD	-0.80	**0.00 HS**

MD: mean difference
CKD (HD): chronic kidney disease on hemodialysis
CKD: chronic kidney disease on conservative treatment

Using T test, no significant difference in serum creatinine of CKD patients on hemodialysis and those on conservative treatment (p= 0.06) as shown in table (3-15).

Table (3-15): Mean serum creatinine with T test between CKD patients on hemodialysis and on conservative treatment.

	Groups	Mean ± SD	SE	Range	T-test	P value
Serum creatinine (mg/dl)	CKD (HD)	8.42 ± 3.41	0.62	3.9 - 20.19	1.89	**0.063 NS**
	CKD	6.51 ± 4.33	0.79	3.1 - 26.47		

CKD (HD): chronic kidney disease on hemodialysis
CKD: chronic kidney disease on conservative treatment

3.3.2. Salivary and blood urea

Figure (3-4) shows the mean blood and salivary urea in CKD patients on hemodialysis and on conservative treatment with salivary urea in control group.

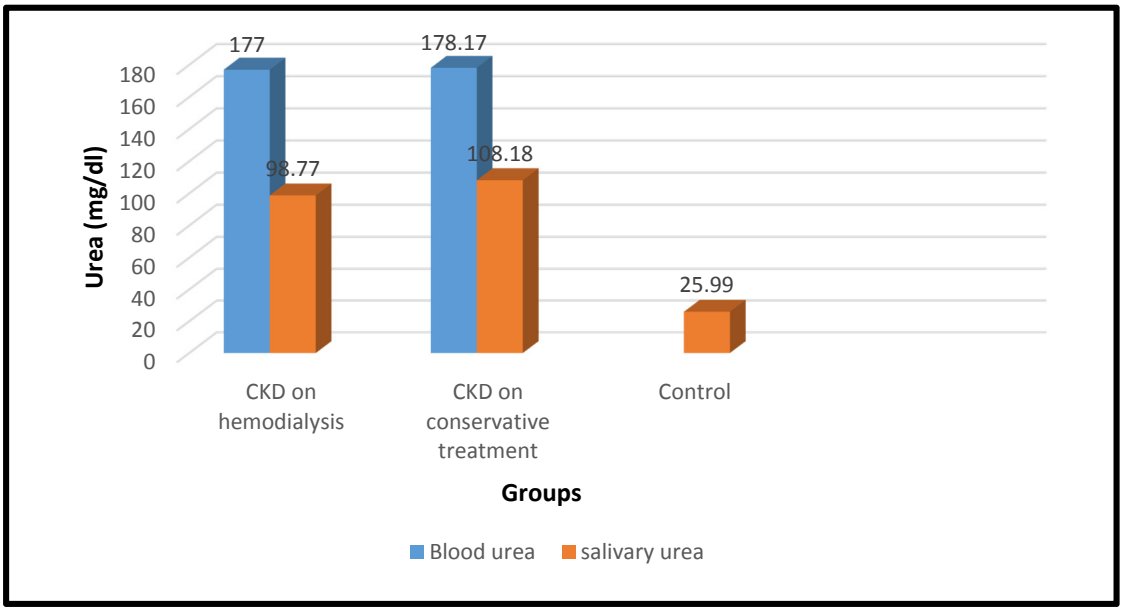


Figure (3-4): Mean blood and salivary urea in CKD patients and control group.

A statistical analysis using ANOVA test showed that there was a significant difference in salivary urea among CKD patients on hemodialysis, those on conservative treatment and control group; table (3-16).

Table (3-16): Mean salivary urea of studied groups with ANOVA test.

	Groups	Mean ± SD	SE	Range	F	P value
Salivary urea (mg/dl)	CKD(HD)	98.77 ± 28.65	5.23	40.21-159.10	151.693	**0.00 S**
	CKD	108.18± 18.20	3.32	72.11-141.60		
	Control	25.99 ± 6.94	1.27	15.31-41.67		

F: ANOVA
CKD (HD): chronic kidney disease on hemodialysis
CKD: chronic kidney disease on conservative treatment

Using a post hoc (Dunnett T3) test, there was a significant increase in salivary urea level in both CKD patients on hemodialysis and those on conservative treatment compared to control group (p= 0.00, p= 0.00). While, there was no significant salivary urea difference was seen between patients on hemodialysis and those on conservative treatment (p= 0.35) as shown in table (3-17).

Table (3-17): Post hoc (Dunnett T3) test of salivary urea among studied groups.

		Group (I)	Group (J)	MD	P value
Salivary urea (mg/dl)	Dunnett T3	CKD (HD)	Control	72.77	**0.00 S**
			CKD	-9.41	**0.35 NS**
		Control	CKD	-82.18	**0.00 S**

MD: mean difference
CKD (HD): chronic kidney disease on hemodialysis
CKD: chronic kidney disease on conservative treatment

Using T-test, there was no significant difference between CKD on hemodialysis and those on conservative treatment regarding blood urea levels (p= 0.9); table (3-18).

Table (3-18): Mean blood urea with T test between CKD patients on hemodialysis and on conservative treatment.

	Groups	Mean ± SD	SE	Range	T-test	P value
Blood urea (mg/dl)	CKD (HD)	177.00 ± 70.34	12.84	96- 450	-0.075	**0.940 NS**
	CKD	178.17 ± 47.53	8.68	101- 268		

CKD (HD): chronic kidney disease on hemodialysis
CKD: chronic kidney disease on conservative treatment

3.3.3. Salivary immunoglobulin A

Using ANOVA test, there was no significant difference in salivary IgA level among CKD patients on hemodialysis, those on conservative treatment and control group (p= 0.3), although the mean salivary IgA was higher in both groups of patients compared to control; table (3-19).

Table (3-19): Mean salivary IgA level in studied groups with ANOVA test.

	Groups	Mean ± SD	SE	Range	F	P value
IgA (µg/ml)	CKD (HD)	232.68 ± 124.42	22.72	33.63- 426.24	1.219	**0.301 NS**
	CKD	234.76 ± 97.28	17.76	70.90- 415.50		
	Control	196.82 ± 93.05	16.99	50.67- 431.46		

F: ANOVA
CKD (HD): chronic kidney disease on hemodialysis
CKD: chronic kidney disease on conservative treatment

3.3.4. Salivary interleukin- 6:

Using ANOVA test, there was a significant difference in salivary interleukin- 6 in CKD patients on hemodialysis, those on conservative treatment and control group as shown in table (3-20).

Table (3-20): Mean salivary interleukin- 6 of studied groups with ANOVA test.

	Group	Mean ± SD	SE	Range	F	P value
IL-6 (ng/L)	CKD (HD)	161.41 ± 71.11	12.98	70.41-349.54	5.530	**0.005 S**
	CKD	146.17 ± 48.98	8.94	76.98- 265.76		
	Control	110.64 ± 59.94	10.94	68.67- 307.87		

F: ANOVA
CKD (HD): chronic kidney disease on hemodialysis
CKD: chronic kidney disease on conservative treatment

Using post hoc (LSD) test, the result shows that there was a significant increase in salivary interleukin- 6 in CKD patients on hemodialysis and those on conservative treatment compared to control group (p= 0.00, p= 0.03). While, there was no significant difference in salivary IL-6 was seen between CKD patients on hemodialysis and those on conservative treatment (p= 0.33) as shown in table (3-21).

Table (3-21): Post hoc (LSD) test of salivary interleukin- 6 among studied groups.

		Group (I)	Group (J)	MD	P value
IL- 6 (ng/L)	LSD	CKD (HD)	Control	50.77	**0.00 S**
			CKD	15.24	**0.33 NS**
		Control	CKD	-35.53	**0.03 S**

MD: mean difference
CKD (HD): chronic kidney disease on hemodialysis
CKD: chronic kidney disease on conservative treatment

3.3.5. Salivary C- reactive protein

Table (3-22) shows that there was a significant difference in salivary CRP in CKD patients on hemodialysis, those on conservative treatment and control group.

Table (3-22): Mean salivary CRP of studied groups with ANOVA test.

	Group	Mean ± SD	SE	Range	F	P value
CRP (mg/l)	CKD (HD)	2.18 ± 0.86	0.16	0.70- 4.58	4.499	**0.014 S**
	CKD	2.20 ± 0.86	0.16	0.87- 3.87		
	Control	1.50 ± 1.32	0.24	0.60- 5.97		

F: ANOVA
CKD (HD): chronic kidney disease on hemodialysis
CKD: chronic kidney disease on conservative treatment

Using post hoc (LSD) test, the result showed that there was a significant increase of salivary CRP in CKD patients on hemodialysis and those on conservative treatment compared to control group (p= 0.01, p= 0.01). While, there was no significant difference was found between salivary CRP in CKD patients on hemodialysis and those on conservative treatment (p= 0.93) as shown in table (3-23).

Table (3-23): Post hoc (LSD) test of salivary CRP among studied groups.

		Group (I)	Group (J)	MD	P value
CRP (mg/l)	LSD	CKD (HD)	Control	0.68	**0.01 S**
			CKD	-0.02	**0.93 NS**
		Control	CKD	-0.71	**0.01 S**

MD: mean difference
CKD (HD): chronic kidney disease on hemodialysis
CKD: chronic kidney disease on conservative treatment

3.4. Correlation coefficient

There was a significant linear positive strong correlation between salivary creatinine and serum creatinine in both CKD patients on hemodialysis (r= 0.770, p= 0.00) and those on conservative treatment (r= 0.932, p= 0.00); Also, a significant positive correlation between salivary urea and blood urea in CKD patients on hemodialysis (r= 0.860. p= 0.00) and those on conservative treatment (r= 0.858, p= 0.00); table (3-24).

Table (3-24): Correlation between salivary creatinine and serum creatinine and salivary urea and blood urea in CKD patients.

Groups	Variable		serum Creatinine	Blood urea
CKD on hemodialysis	Salivary creatinine	r	0.770	
		P	0.000	
	Salivary urea	r		**0.860**
		P		**0.000**
CKD on conservative treatment	Salivary creatinine	r	0.932	
		P	0.000	
	Salivary urea	r		**0.858**
		P		**0.000**

Also, a significant positive correlation was found between salivary IL-6 and CRP in CKD patients on hemodialysis (r= 0.781, p=0.00), those on conservative treatment (r= 0.840, p= 0.00) and in control group (r= 0.816, p= 0.00) as shown in table (3-25).

Table (3-25): Correlation between salivary IL-6 and CRP in CKD patients and control group.

Group			CRP
CKD on hemodialysis	IL-6	r	**0.781**
		P	**0.000**
CKD on conservative treatment	IL-6	r	**0.840**
		P	**0.000**
Control	IL-6	r	**0.816**
		P	**0.000**

Considering salivary flow rate and oral manifestations, no significant correlation between salivary flow rate and oral manifestations in both patients groups, except dry mouth; a significant negative correlation was seen in both CKD patients on hemodialysis (r=-0.762, p= 0.00) and those on conservative treatment (r= -0.722, p= 0.00); table (3-26).

Table (3-26): Correlation between salivary flow rate and oral manifestations in CKD patients.

		Salivary flow rate	
Groups	Oral manifestations	r	p
CKD on hemodialysis	Dry mouth	-0.762	**0.00 HS**
	Uremic fetor	-0.112	**0.554**
	Taste change	0.080	**0.676**
	Pale oral mucosa	-0.033	**0.863**
	Aphthus ulceration	0.142	**0.454**
	Gingival enlargement	0.227	**0.227**
	Burning sensation	0.049	**0.797**
	Angular cheilitis	-0.139	**0.465**
CKD on conservative treatment	Dry mouth	-0.722	**0.00**
	Uremic fetor	-0.103	**0.589**
	Taste change	0.163	**0.388**
	Pale oral mucosa	-0.133	**0.484**
	Aphthus ulceration	0.123	**0.516**
	Gingival enlargement	-0.186	**0.325**
	Burning sensation	-0.322	**0.082**
	Angular cheilitis	0.007	**0.973**

HS: highly significant

CHAPTER FOUR

DISCUSSION

Discussion

4.1. Clinical findings

4.1.1. Demographic findings

4.1.1.1. Age and gender

This study showed that the mean age of CKD patients on hemodialysis was 55.3 years, while the mean age of CKD with conservative treatment was 49.3 years. This agrees with Denic *et al*., (2016) who explained that the kidney function decline with age.

Considering gender, males were more than females in both patients groups. In CKD patients on hemodialysis, 17 were males and 13 were females, while in CKD patients with conservative treatment, 16 were males and 14 were females. This result is in agreement with Goldberg and Krause (2016) who found that a higher progression rate and mortality risk was seen in CKD males patients compared to females, while this result inconsistent with Ahmed *et al*., study in (2015) who reported that females patients was more than males patients.

Sex hormones are thought to play an important role in the biological mechanisms associated with variability in CKD prevalence and characteristics between males and females. Animal studies have been demonstrated the harmful effect of testosterone and protective effect of estrogen on several biological processes that are involved in renal damage. However, the role of sex hormones in clarifying gender-related differences in CKD in humans has not yet been established (Goldberg and Krause, 2016).

In this study, there was no significant differences among the studied groups regarding age and gender. This result parallel with other studies done previously (Bello *et al*., 2008; Bader *et al*. 2015; Khozeymeh *et al*., 2016).

4.1.1.2. Occupation and marital status

In the current study, most of CKD patients on hemodialysis were unemployed (13) followed by retired (9), officer (4) and worker (4). While those on conservative treatment, the majority were retired (14) followed by worker (10), unemployed (3) and officer (3).

In relation to marital status, the majority of CKD patients on hemodialysis were married (27) followed by single (3). In those on conservative treatment, (19) were married followed by single (6), widowed (3) and divorced (2).

There was no significant difference among studied groups considering to occupation and marital status. This result coincide with a study done by Huda *et al*., (2012) for occupation, while it disagrees with the same study in relation to marital status. Another study done by Pinho *et al*., (2015) found that there was a significant association between CKD and marital status, which disagrees with the current study regarding to marital status.

Living with a partner has been considered a family support indicator, which would be associated with better treatment commitment by CKD patients and better health outcomes (Maldaner *et al*., 2008; Pierin *et al*., 2010). This positive effect of living with a partner was not observed in the current study, which may be due to different population circumstances.

4.1.2. Salivary flow rate and PH

4.1.2.1. Salivary flow rate

In this study, there was a significant difference among studied groups regarding salivary flow rate.

Although the mean salivary flow rate was lower in hemodialysis patients compared to control group, no statistically significant difference

between CKD patients on hemodialysis and control group. This may be due to sample size or types of patients.

Several studies reported that there was a significant lowering of salivary flow rate in hemodialysis patients compared to control (Kaushik *et al*., 2013; Anuradha *et al*., 2015; Hatem and Mohammad, 2015) which is in agreement with current study.

A significant lower salivary flow rate in CKD patients on conservative treatment compared to control group was seen. This is in agreement with other studies that found a significant decrease in salivary flow rate in CKD patients compared to control group (Belazelkovska *et al*., 2014; Oyetola *et al*., 2015).

However, a study done by Ersson *et al*. (2011) found no significant difference between predialysis patients and control group in salivary flow rate, which is inconsistent with the finding of the current study.

Lower salivary flow rates attributed to direct uremic involvement of the salivary glands leading to reduced parenchymatous and excretory functions, and as a result of dehydration due to fluid intake restriction. Acute stress levels in those patients may also decrease the salivary flow rate according to Kaushik *et al*., (2013).

4.1.2.2. Salivary PH

In this study, there was a significant difference in salivary PH among studied groups.

A significant higher salivary PH in both patients groups when compared to control group. This agrees with previous studies (Al Nowaiser *et al*., 2003; Honarmand *et al*., 2017). However, it is inconsistent with another study done by Belazelkovska *et al*. (2014) who found that there was no significant difference in salivary PH between patients and control.

This may be explained by urease, salivary urea is decomposed into ammonium ions and carbon dioxide that might raise salivary pH to critical levels in those patients (Al Nowaiser *et al*., 2003; Honarmand *et al*., 2017).

4.1.3. Smoking status:

The results of the current study revealed that the majority of CKD patients on hemodialysis were non-smokers (26) followed by ex-smokers (3) and current smoker (1). Similarly, patients on conservative treatment were mainly nonsmokers (22) followed by current smokers (6) and ex-smokers (2).

Regarding smoking and numbers of cigarette per day, no significant difference among studied groups which is inconsistent with a study done by Yacoub *et al*., (2010) who found that current smokers were under an increased risk of having CKD compared to nonsmokers.

Many studies explained that smoking as a risk factor for progression of CKD (Orth and Hallan, 2008; Nagasawa *et al*., 2012; Júnior *et al*., 2014).

4.1.4. Medical history

In this study, the majority of patients were with either diabetic or hypertension. In CKD patients on hemodialysis, the majority were with hypertension, while those on conservative treatment, diabetic mellitus was the most common, however Chi square test was non-significant.

This is in agreement with other studies done by Lea and Nicholas (2002) and Suleymanlar *et al*. (2011) who reported that diabetes mellitus and hypertension were common among CKD patients. However, this disagrees with another study done by Kabir *et al*., (2012) who found that there was a significant difference between cases and control regarding diabetic mellitus and hypertension.

Diabetes mellitus and hypertension may be considered as important causes of CKD; therefore, the international guidelines recommend yearly screening for CKD in diabetic or hypertensive patients (van der Meer *et al*., 2010). Uncontrolled diabetes and/or hypertension can easily and quickly progress to end stage renal disease (Kazancioğlu, 2013).

4.1.5. Family history

In the current study, the majority of patients in both patients groups; on hemodialysis and on conservative treatment were with 1st degree relative family history of CKD.

No significant difference among studied groups in relation to 1st and 2nd family history. This is parallel with a study done by Kabir *et al*., (2012) who found no significant difference regarding risk factor like family history. But, inconsistent with another study done by Orantes *et al*., (2011) who found that developing CKD is significantly influencing by family history of CKD.

Family history of kidney disease is one of the crucial risk factor for CKD. Therefore, it is advisable to screen high-risk family members of CKD patients to prevent the disease (Kazancioğlu, 2013).

4.2. Oral findings

In this study, many oral findings were seen in both patients' groups.

Dry mouth was seen in 46.67% of CKD patients on hemodialysis, this is in agreement with Honarmand *et al*. (2017) who observed 46.7% of hemodialysis patients with xerostomia, but lower than that reported in study conducted by Patil *et al*. (2012) who found 91% of patients were with dry mouth.

In CKD patients on conservative treatment, 36.6% was found with dry mouth, this is lower than that reported by Belazelkovska *et al*. (2013) who fonud 73.3% of patients were with dry mouth.

Dry mouth may be caused by direct gland involvement, fluid restriction, use of medications and mouth breathing (Ahmed *et al*., 2015).

Regarding uremic fetor, 50% of CKD patients on hemodialysis were with uremic fetor. This is almost similar to other studies (de la Rosa García *et al*., 2006; Ali *et al*., 2015) that mentioned 48.5% and 45% of patients were with uremic breath, respectively.

A study done by Ahmed *et al*. (2015) reported that 66 % of patients were with uremic fetor which is more than what was found in current study.

Patients on conservative treatment, 33.3% were with uremic fetor which is more than that reported by Belazelkovska *et al*. (2013) who was observed 26.6% of patients with uremic fetor.

Uremic fetor, an ammoniacal odor typical of uremic patients, caused by high urea level in the saliva which is decomposed to ammonia by urease. In addition, oral malodor also result from neglected oral hygiene due to the chronic nature of the disease (Kaushik *et al*., 2013).

In relation to taste change, 43.3% of CKD patients on hemodialysis suffered from taste change. This is almost similar to study done by de la Rosa García *et al*. (2006) who reported 45.5% of patients were with unpleasant taste and exactly similar to Honarmand *et al*. (2017) who observed 43.3% of patients complaining from altered taste.

In those on conservative treatment, 46.6% were with taste change, which is more than that reported by other studies (Belazelkovska *et al*., 2013; Oyetola *et al*., 2015) which was 26.66% and 26%, respectively.

Metallic taste in uremic patients has been reported to be due to urea concentration in the saliva and its subsequent decomposition to ammonia and carbon dioxide by urease. The taste change can also be due to the use of medication, metabolic disturbance, diminished taste buds number and changes in the salivary flow and composition. Another study reported that

high urea levels, dimethyl and trimethyl amines and low levels of zinc might be associated with decreased taste perception in uremic patients (Asha *et al*., 2012; Kuravatti *et al*., 2016).

Concerning pale oral mucosa, 40% of CKD patients on hemodialysis were with pale oral mucosa. This was almost similar to what was reported in study done by Honarmand *et al*. (2017) who found 42.2% of patients were with pale oral mucosa. Patil *et al*. (2012) mentioned that 87% of patients were with pale oral mucosa, this is more than twice the findings of current study.

In those on conservative treatment, 20% were with pale oral mucosa. This is almost similar to study done by Oyetola *et al*. (2015) who found 24% and half in frequency than in a study done by Belazelkovska *et al*. (2013) who found that 53.3% of patients were with from mucosal pallor.

Pale oral mucosa secondary to anemia mainly developed due to inability of the failing kidneys to produce erythropoietin, loss of red blood cells through dialysis, reduced red blood cells survival time and their early destruction and, in some cases, from malnutrition (Anuradha *et al*., 2015; Honarmand *et al*., 2017).

Regarding aphthus ulceration, 23.3% of CKD patients on hemodialysis complained from aphthus ulceration, which is more than those reported in a study done by Ahmed *et al*. (2015) who reported 2.8%.

In those on conservative treatment, 16.6% were with aphthus ulceration. This is more than those mentioned in study performed by Oyetola *et al*. (2015) who reported 2%.

It is well known that aphthus ulcers may occur due to psychological stress of CKD patients especially those on hemodialysis,

impaired immune system or nutritional problems such as folic acid or iron deficiency.

Concerning gingival enlargement, 13.3% of CKD patients on hemodialysis were with gingival enlargement in this study. Ahmed *et al*. (2015) found that 33.9% of hemodialysis patients were with gingival enlargement which is more than those in current study.

While in those on conservative treatment, 16.6 % of patients complained from gingival enlargement. This is inconsistent with Belazelkovska *et al*. (2013) who reported that there was no CKD patients on conservative treatment with gingival enlargement.

Such enlargement can be induced by calcium channel blockers (nifedipine, amlodipine, verapamil, diltiazem) used for management of hypertension in CKD patients. The condition in turn may be aggravated by the neglected oral care **(**Kaushik *et al*., 2013).

In relation to burning sensation, 6.6% of hemodialysis patients suffered from burning sensation. This is lower than those mentioned in a study done by Ahmed *et al*. (2015) who reported 25.7%.

In those on conservative treatment, 10% were with burning sensation, this finding disagrees with Belazelkovska *et al*. (2013) who reported that there was no patients on conservative treatment complained from burning sensation.

Predominant reasons for the complaining of burning sensation are xerostomia, the presence of candidiasis, prolonged clearance of medications as well as vitamin deficiency (Belazelkovska *et al*., 2013).

Regarding of angular cheilitis, 3.3% of hemodialysis patients complained from angular cheilitis. This is almost similar to that reported in study conducted by Murali *et al*. (2012) who found 5%. Another study done by Ali *et al*. (2015) found 29% of patients were with angular cheilitis.

In those on conservative treatment, also 3.3% were with angular cheilitis. Belazelkovska *et al*. (2013) found 46.6% of patients suffered from angular cheilitis which is much more than those in the current study. It has been found that angular cheilitis associated with candida infection and anemia (Ahmed *et al*., 2015; Oza and Doshi, 2017).

No significant difference between CKD patients on hemodialysis and those on conservative treatment regarding all oral manifestations.

A few studies investigating oral manifestations in CKD patients on different treatment modality.

Belazelkovska *et al*. (2013) reported that there was a significant difference between hemodialysis patients and those on conservative treatment concerning uremic fetor, burning sensation and pale oral mucosa. This is inconsistent with the findings of the current study.

4.3. Laboratory findings

4.3.1. Salivary and serum creatinine and urea

In the current study, a significant difference in salivary creatinine and urea among studied groups was seen. There was a significant increase in salivary creatinine and urea levels in both patients groups compared to control group. This is in agreement with other studies which reported that a salivary creatinine and urea level in CKD patients is higher compared to control group (Venkatapathy *et al*., 2014; Bader *et al*., 2015; Lasisi *et al*., 2016; Yajamanam *et al*., 2016; Bagalad *et al*., 2017), While no significant difference between the two patients groups regarding both salivary creatinine and urea.

Also, no significant difference in serum creatinine and urea between the two patients groups was reported.

Increased salivary concentration may be due to increased serum concentration which creates a concentration gradient which in turn increases the diffusion of creatinine and urea from serum to saliva in

CKD patients. It is also possible that high level of creatinine and urea in saliva may be an alternative route of excretion in compromised kidney function state (Nakahari *et al*., 1996; Chand *et al*., 2018).

4.3.2. Salivary Immunoglobulin A

In the present study, mean salivary IgA was higher in both patients groups compared to control group, although statistically no significant difference among studied groups was found. This could be attributed to the differences in the sample size and patient factors.

Current study finding is in the line with other studies, which reported a significantly higher level of salivary IgA in CKD patients compared to healthy subjects (Hatem and Mohammad, 2015; *Konstantinova et al*., 2017; Staykova *et al*., 2018). The statistical difference between the present study and other previous studies may be attributed to the sample size, laboratory tests and patients factors.

4.3.3. Salivary interleukin-6

This study showed a significant difference in salivary IL-6 level among studied groups. However, no significant salivary IL-6 difference between two patients groups.

A significant higher level of salivary IL-6 in CKD patients on hemodialysis compared to control group was seen. This finding is consistent with a study done by Khozeymeh *et al*. (2016) who reported a significant increase in salivary IL-6 level in hemodialysis patients compared to control subjects.

Also, a significant increase in salivary IL-6 in CKD patients on conservative treatment compared to control group was found. This is consistent with Ersson *et al*. (2011) who reported a higher level of salivary IL-6 in CKD patients compared to control subjects, but unfortunately it was measured in a limited number of patients which was non statistically significant.

Increased inflammatory biomarkers levels in CKD can promote atherosclerosis and thrombosis (Shlipak *et al*., 2003). These mechanisms may explain the high prevalence of cardiovascular disease among CKD patients. Therefore, measurement of cytokines levels in saliva may be considered as a noninvasive test for cardiac risk stratification in hemodialysis patients (Khozeymeh *et al*., 2016).

Many oral diseases including oral cancer, lichen planus and periodontal diseases have been reported to be associated with IL-6 deregulation (Nibali *et al*., 2012). Periodontal diseases are prevalent in patients on hemodialysis who showed a bad oral care and its prevalence increases with the chronicity of the disease (Hamissi *et al*., 2009). The important role of IL-6 in the loss of periodontal ligament and alveolar bone through tissue degradation effects of IL-6 on connective tissue and bone, mediated by metalloproteinase and osteoclasts activity (Nibali *et al*., 2012).

Up to our knowledge, few studies were performed to investigate the salivary IL-6 level in CKD patients, so further studies may be need to confirm the pathological role of IL-6 in CKD patients.

4.3.4. Salivary C-reactive protein

In the current study, there was a significant salivary CRP difference among studied group. However, there was no significant difference in salivary CRP level between the two patients groups.

A significant higher salivary CRP level in hemodialysis patients and those on conservative treatment compared to control group was seen.

Pallos *et al*. (2015) found that there was a significant higher level of salivary CRP in patients on hemodialysis compared to normal subjects while no significant difference between those on conservative treatment and normal subjects. This finding agrees with the current study regarding

hemodialysis patients, but disagrees with the findings of conservative treated patients.

A few studies regarding CRP in saliva of patients with CKD. However, several studies measured it in the serum of CKD patients and found that a significant increase in CRP level in serum of patients with CKD (Fox *et al*., 2010; Ersson *et al*., 2011; Adejumo *et al*., 2016).

Importantly, periodontal disease can worsen CKD. A systemic review and meta-analysis reported an increased prevalence of CKD in patients with periodontitis (Chambrone *et al*., 2013).

Many studies have proved a positive association between the presence of chronic periodontitis and high level of serum CRP (Slade *et al*., 2003; Gomes-Filho *et al*., 2011; Goyal *et al*., 2014).

Inflammatory cytokines (IL-6, IL-1 and TNF-α) released in a response to periodontal infection and stimulate hepatocytes to produce CRP. Therefore, in the presence of chronic periodontitis, higher serum CRP levels would be found (Bansal *et al*., 2014).

4.4. Correlation coefficient

The present study showed that there was a significant positive correlation between salivary creatinine and urea and serum creatinine and urea levels, respectively in both patients groups. This is in agreement with other studies which reported that the levels of creatinine and urea in both saliva and serum were positively correlated in CKD patients (Bader *et al*., 2015; Lasisi *et al*., 2016; Pandya *et al*., 2016; Yajamanam *et al*., 2016; Bagalad *et al*., 2017; Chand *et al*., 2018).

This study supported the fact that whenever there was an increase in serum creatinine and blood urea, there would be concomitant increase in salivary creatinine and urea also.

Also, a significant positive correlation between salivary IL-6 and CRP in CKD patients and control group. IL-6 is known to induce the

production of CRP in the liver (Eklund, 2009). Therefore, as expected, the levels of IL-6 and CRP were positively correlated in this study.

While no significant correlations between salivary flow rate and oral findings, except the correlation between salivary flow rate and dry mouth which is negative significant correlation in both patients groups. Belazelkovska *et al*. (2014) reported that a significant negative correlation was found between unstimulated salivary flow rate and xerostomia in CKD patients on hemodialysis and those on conservative treatment. This was quite similar to what was seen in current study.

Xerostomia is a subjective symptom of dry mouth (this could be due to qualitative salivary change). It is a sensation that is assessed only by direct questioning the person. On the other hand, the objective sign of dry mouth is salivary gland hypofunction resulting in reduction of the quantity of saliva (Joanna and Thomson, 2015). The significantly reduced mean salivary flow rate in CKD patients can be a contributory factor to xerostomia (Kaushik *et al*., 2013).

CHAPTER FIVE

CONCLUSIONS & SUGGESTIONS

Conclusions and Suggestions

5.1. Conclusions

1 Dry mouth, uremic fetor and oral taste changes are the most common oral findings in both CKD patients on hemodialysis and those on conservative treatment. Pale oral mucosa, aphthus ulceration, gingival enlargement, burning sensation and angular cheilitis are also seen.

2. Salivary flow rate is lower in both hemodialysis and conservatively treated patients compared to healthy subjects.

3. Salivary PH is higher in both groups of patients compared to healthy subjects.

4. Salivary creatinine and urea levels is higher in both groups of patients compared to healthy subjects.

5. A significant positive correlations was found between both salivary and serum creatinine and salivary and blood urea in CKD patients.

6. Immunological markers (salivary IgA, IL-6 and CRP) demonstrated a difference in both groups of patients compared to healthy subjects.

7. There was a significant positive correlation between salivary IL-6 and CRP in CKD patients.

8. Patients with CKD need comprehensive strict professional oral care and self-care instructions.

5.2. Suggestions

1. A longitudinal study of oral manifestations in a large numbers of CKD patients.
2. Study to compare IgA, IL-6 and CRP level in serum and saliva of CKD patients on hemodialysis.
3. Study to assess the salivary IgA, IL-6 and CRP levels in hemodialysis, peritoneal and transplanted patients.
4. Study to compare salivary IL-6 and CRP levels in CKD patients with and without cardiovascular diseases.

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APPENDICES

Appendix 1

موافقة للاشتراك في البحث العلمي

اسم الباحث:

عنوان البحث:

مكان اجراء البحث:

انت مدعو(ة) للمشاركة ببحث علمي سريري سيجرى في.....

الرجاء ان تأخذ(ي) الوقت الكافي لقراءة المعلومات التالية بتأن قبل ان تقرر(ي) اذا كنت تريد(ين) المشاركة ام لا. بامكانك طلب ايضاحات او معلومات اضافية عن اي شي مذكور في هذه الاستمارة اة عن هذه الدراسة ككل من طبيبك.

في حال وافقت عن المشاركة في هذه الدراسة ، سيبقى اسمك طي الكتمان. لن يكن لأي شخص ، مالم ينص القانون على ذلك، حق الاطلاع على ملفك الطبي باستثناء الطبيب المسؤول عن الدراسه ومعاونيه.

موافقة المشترك:

لقد قرأت استمارة القبول هذه وفهمت مضمونها. تمت الاجابة على اسئلتي جميعها. وبناء عليه فأنني ، حرا مختارا ، اجيز اجراء هذا البحث واوافق على الاشتراك فيه واني اعلم ان الباحثة الدكتورة ايثار كريم سالم وزملاءها ومعاونيها او مساعديها سيكونون مستعدين للاجابة على اسئلتي ، وان بأستطاعتي الاتصال بهم على الهاتف ..... واذا شعرت لاحقا ان الاجوبة تحتاج الى مزيد من الايضاح فسوف اتصل باحد اعضاء لجنة الاخلاقيات. كما اعرف تمام المعرفة بأنني حر في الانسحاب من هذا البحث متى شأت حتى بعد التوقيع على الموافقة دون ان يأثر ذلك على العناية الطبية المقدمة لي.

اسم المشترك:

توقيع المشترك:

التاريخ:

Appendix 2

University of Baghdad

College of dentistry

Oral medicine department

Case number:

Date: / /

- Patient's demography…

Patient name: physician name:

Gender: age: address:

Occupation: phone number:

Marital state: married ☐ divorced ☐ widow ☐ single ☐

- Risk factors…

Smoking: no ☐ yes ☐ no. of cigarette/day:

Alcohol consumption:

- Family history:

- Medical history:

-cardiovascular disease	yes	no
-respiratory disease	yes	no
-genitourinary disease	yes	no
-gastrointestinal disease	yes	no
-Hematological disease	yes	no
-Endocrine disease	yes	no
-Neurological disease	yes	no
-Musculoskeletal disease	yes	no
-Dermatological disease	yes	no
-Immunological disease	yes	no

-Infectious disease yes no

-Surgery or hospitalization yes no

-Pregnant or nursing (women) yes no

-Medication yes no ……..

-Allergy yes no

- History of present illness:

- Investigations:

Serum creatinine: blood urea:

- Oral manifestation:

Uremic fetor yes no

Taste change yes no

Dry mouth yes no

Burning sensation yes no

Pale oral mucosa yes no

Mucosal petechiae and ecchymosis yes no

Uremic stomatitis yes no

Candidiasis yes no

Aphthus ulceration yes no

Angular chelitis yes no

Gingival enlargement yes no

Enamel hypoplasia yes no

- Salivary Parameters:

Salivary flow rate (ml/min): PH:

Appendix 3:

Secretory IgA kit

•Contents of the kits

Items	Quantity
secretory IgA EIA strips, 8x12 wells	1
Calibrator set, 1 ml each. The set contains 6 calibrators: 0; 2; 20; 40; 100, 400 µg/ml	6
Control serum (1 ml)	1
Conjugate, 11 ml	1
red EIA buffer 22 ml	1
EIA buffer 100 ml	1
Substrate solution, 11 ml	1
Washing solution concentrate 21x, 22 ml	1
Stop solution, 11 ml	1
Plate sealing tape	2
Instruction secretory IgA EIA	1
QC data sheet secretory IgA EIA	1

• Reagent Preparation

- All reagents (including unsealed microstrips) should be allowed to reach room temperature (+18 to +25 °C) before use.

- All reagents should be mixed by gentle inversion or vortexing prior to use. Avoid foam formation.

- It is recommended to spin down shortly the tubes with calibrators on low speed centrifuge.

- Prepare washing solution from the concentrate BUF WASH 21X by 21 dilutions in distilled water.

• Calculation of results

- Calculate the mean absorbance values (OD450) for each pair of calibrators and samples.

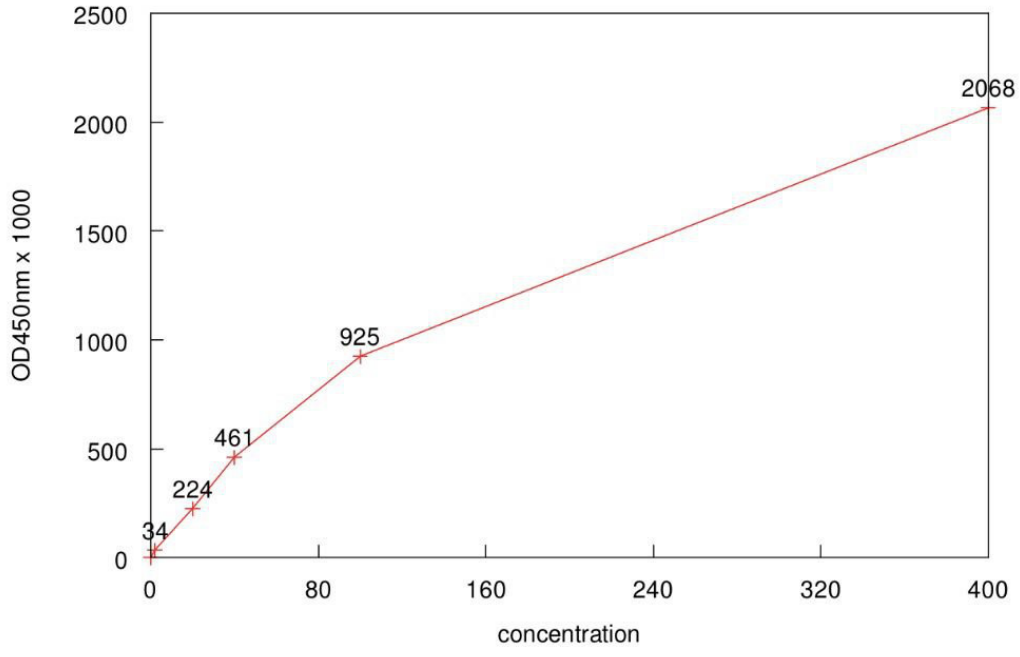
- Plot a calibration curve on graph paper: OD versus secretory IgA concentration.

- Determine the corresponding concentration of secretory IgA in unknown samples from the calibration curve. Manual or computerized data reduction

is applicable on this stage. Point-by-point or linear data reduction is recommended due to non-linear shape of curve.

- Below is presented a typical example of a standard curve with the Demeditec Assay. Not for calculations.

Calibrators	Value	Absorbance Units (450 nm)
CAL 1	0 µg/ml	0.10
CAL 2	2 µg/ml	0.14
CAL 3	20 µg/ml	0.33
CAL 4	40 µg/ml	0.57
CAL 5	100 µg/ml	1.03
CAL 6	400 µg/ml	2.17



Appendix 4:

Interleukin-6 kit

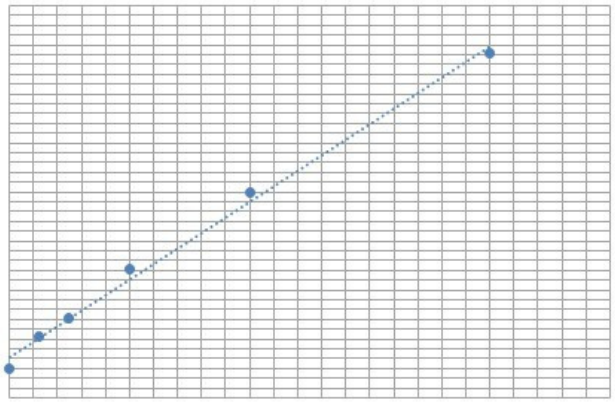
•Contents of the kit

Items	Quantity
Instruction	1
Seal plate membrane	2
Hermetic bag	1
Coated ELISA plate	12-well * 8 tubes
Standard solution(640ng/L)	0.5ml×1
Streptavidin-HRP	6ml×1
Stop Solution	6ml×1
chromogenic reagent A	6ml×1
chromogenic reagent B	6ml×1
Anti IL-6 antibodies labeled with biotin	1ml×1
Standard dilution	3ml×1
Washing concentrate	(20ml×30)×1

• Hold kit at room temperature for at least 30 minutes once removed from 2-8℃ environment.

•Calculation of results

Make concentration of standards the abscissa and OD value the ordinate. Draw the standard curve on the graph paper. According to the OD value of the sample, locate its corresponding concentration (which the concentration of the sample); or calculate the linear regression equation of the standard curve according to the standard concentration and the OD value. Then substitute with the OD value of the sample to calculate its concentration.



the chart is for reference only

Appendix 5:

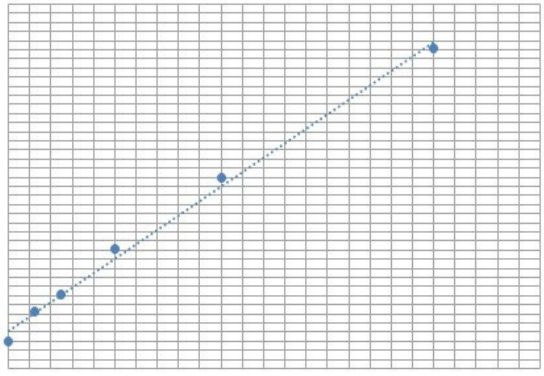
C-reactive protein kit

• Contents of the kit

Items	Quantity
Instruction	1
Seal plate membrane	2
Hermetic bag	1
Coated ELISA plate	12-well * 8 tubes
Standard solution(7.2mg/L)	0.5ml×1
Streptavidin-HRP	6ml×1
Stop Solution	6ml×1
chromogenic reagent A	6ml×1
chromogenic reagent B	6ml×1
Anti CRP antibodies labeled with biotin	1ml×1
Standard dilution	3ml×1
Washing concentrate	(20ml×30)×1

• Hold kit at room temperature for at least 30 minutes once removed from 2-8℃ environment.

• Calculation of results



the chart is for reference only

Make concentration of standards the abscissa and OD value the ordinate. Draw the standard curve on the graph paper. According to the OD value of the sample, locate its corresponding concentration (which is the concentration of the sample); or calculate the linear regression equation of the standard curve according to the standard concentration and the OD value. Then substitute with the OD value of the sample to calculate its concentration**.**

الخلاصة

خلفية الدراسة:
مرض الكلى المزمن هو خسارة تدريجية في وظائف الكلى على مدى أشهر أو سنوات ويعتبر مرض السكري وارتفاع ضغط الدم كسبب رئيسي. وينقسم المرض إلى خمس مراحل. وتسمى المرحلة الأخيرة بالفشل الكلوي او الداء الكلوي بمراحله الأخيرة التي تصبح مميتة في غياب الغسل الكلوي أو زراعة الكلى.
ويرتبط الالتهاب المزمن الملاحظ في المرض بزيادة مستويات المصل من مفاعلات بروتينات المرحلة الحادة ، مثل بروتين سي التفاعلي ، ومجموعة متنوعة من وسطاء الالتهابات المناعية ، مثل السيتوكينات. انترلوكين واحد ، انترلوكين ستة وعامل نخر الورم الفا هي من بين السيتوكينات المعززة للالتهاب التي ترتبط بالفيزيولوجيا المرضية لمرض الكلى. هناك دليل على التنشيط المناعي في المراحل المبكرة من مرض الكلى المزمن في الأشخاص البالغين. كما تم الكشف عن وجود ارتباط بين مستويات بروتين سي التفاعلي والسيتوكينات المعززة للالتهاب ، ولا سيما الانترلوكين السادس.
مرض الكلى المزمن هو واحد من الأمراض الجهازية التي يمكن أن تؤثر على محتويات إفراز اللعاب. يمكن أن يشير اللعاب إلى مستويات الكرياتينين واليوريا في مرضى الكلى المزمن ، وهي المعلمات المقاسة عمومًا في عينات الدم.
اهداف الدراسة:
تهدف هذه الدراسة إلى تحديد الظواهر الفموية ، قياس معدل تدفق اللعاب و الرقم الهيدروجيني له وتحديد الغيوبولين المناعي، انترلوكين ستة، بروتين سي التفاعلي وعلامات وظائف الكلى (الكرياتينين واليوريا) في لعاب مرضى الكلى المزمن الخاضعين للغسل الكلوي والمرضى الذين هم على العلاج المحافظ والمقارنة مع الاحصاء. أيضا لتقييم إمكانية استخدام اللعاب كسوائل التشخيص من خلال مقارنة مستوى علامات وظيفة الكلى في اللعاب ودم المرضى الذين يعانون من مرض الكلى المزمن.
المواد وطرائق العمل:
تضمنت هذه الدراسة تسعين شخصا وتم تقسيمهم الى ثلاث مجموعات: 30 مريضا يعانون من مرض الكلى المزمن الخاضعين للغسل الكلوي لمدة 6 أشهر على الأقل ؛ 30 مريضا يعانون من مرض الكلى المزمن والذين هم على العلاج المحافظ و 30 مشاركا من الاصحاء. تم إجراء فحص فموي لكل مشارك مع تسجيل المظاهر الفموية. تم جمع اللعاب وحساب معدل تدفق اللعاب ملليلتر في الدقيقة وتم قياس الرقم الهيدروجيني بواسطة مقياس الرقم الهيدروجيني الرقمي. تم قياس الغلوبيولين المناعي، انترلوكين ستة وبروتين سي التفاعلي في عينات اللعاب بواسطة ELISA.
وتم تقدير مستوى الكرياتينين في عينات اللعاب عن طريق الطريقة اللونية وتم قياس مستوى اليوريا اللعابي بواسطة محلل روش - كوباس سي 111 تلقائيا.
النتائج:
كان جفاف الفم ، التنفس اليوريمي وتغير المذاق من أكثر المظاهر الفموية شيوعا في المرضى الذين يعانون من مرض الكلى المزمن الخاضعين للغسل الكلوي والمرضى الذين هم على العلاج المحافظ. الغشاء المخاطي الفموي الباهت ، تقرح الفم ، تضخم اللثة ، الإحساس بالحرقان والشفة الزاويّة وجدت ايضا في المرضى. لم يتم العثور على فروق ذات دلالة إحصائية في المظاهر الفموية بين المجموعتين من المرضى.
كان معدل تدفق اللعاب أقل في المجموعتين من المرضى مقارنة بالاصحاء. فيما يتعلق بالرقم الهيدروجيني اللعابي ، الرقم الهيدروجيني اللعابي أعلى في المرضى المصابين بمرض الكلى المزمن الخاضعين للغسل الكلوي و المرضى الذين هم على العلاج المحافظ مقارنة مع الاصحاء.

كان مستوى الغلوبيولين المناعي اعلى في لعاب المرضى المصابين بمرض الكلى المزمن الخاضعين للغسل الكلوي والمرضى الذين هم على العلاج المحافظ مقارنة مع مجموعة الاصحاء على الرغم من عدم وجود دلالة احصائية.
كانت هناك زيادة ملحوظة في الانترلوكين ستة و بروتين سي التفاعلي في لعاب المرضى المصابين بمرض الكلى المزمن الخاضعين للغسل الكلوي والمرضى الذين هم على العلاج المحافظ مقارنة مع مجموعة الاصحاء. تم العثور على ارتباط إيجابي كبير بين انترلوكين ستة وبروتين سي التفاعلي في لعاب المرضى المصابين بمرض الكلى المزمن الخاضعين للغسل الكلوي (r = 0.781، p = 0.00) ، والذين هم على العلاج المحافظ (r = 0.840، p = 0.00) وفي مجموعة الاصحاء (r = 0.816، p = 0.00).
أيضا ، كان هناك زيادة كبيرة في مستويات الكرياتينين واليوريا في اللعاب لكل من مرضى الكلى المزمن الخاضعين للغسل الكلوي والذين هم على العلاج المحافظ مقارنة مع مجموعة الاصحاء. فيما يتعلق بمستوى الكرياتينين واليوريا في المصل ، لم يُشاهد أي فرق كبير بين المجموعتين من المرضى. هناك ارتباط إيجابي كبير بين الكرياتينين اللعابي والكرياتينين في المصل في المرضى المصابين بمرض الكلى المزمن الخاضعين للغسل الكلوي (r = 0.770، p = 0.00) والذين هم على العلاج المحافظ (r = 0.932، p = 0.00). أيضا ، وجود علاقة إيجابية كبيرة بين اليوريا اللعابية واليوريا في الدم في مرضى الكلى المزمن الخاضعين للغسل الكلوي (r= 0.860 ، P = 0.00) والذين هم على العلاج المحافظ (r= 0.858 ، P = 0.00).

الاستنتاجات:

هناك مظاهر فموية شائعة في المرضى المصابين بمرض الكلى المزمن. معدل تدفق اللعاب أقل في المرضى الذين يعانون من مرض الكلى المزمن مقارنة مع الاصحاء. الرقم الهيدروجيني اللعابي ، الغلوبيولين المناعي، انترلوكين ستة، بروتين سي التفاعلي ، الكرياتينين واليوريا أعلى في لعاب المرضى المصابين بمرض الكلى المزمن مقارنة مع الاصحاء.

وزارة التعليم العالي
والبحث العلمي
جامعة بغداد
كلية طب الاسنان

الظواهر الفموية، عامل الغلوبيولين المناعي (A)، انترلوكين- ستة، بروتين سي التفاعلي ومؤشرات وظائف الكلى في لعاب المرضى المصابين بمرض الكلى المزمن

رسالة

مقدمة الى مجلس كلية طب الاسنان في جامعة بغداد وهي جزء من متطلبات درجة الماجستير في علم طب الفم

مقدم الرسالة

ايثار كريم سالم

بكالوريوس طب وجراحة الفم والاسنان

اشراف

أ.م.د. امينة ريحان دعيجل

دكتوراه في طب الفم

1440 هـ

2018 م