Ministry of Higher Education

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University of Babylon

College of Medicine

Study of Some Serum Biomarkers and Salivary Functional Groups in Patients with Renal Impairment

A Thesis

Submitted to the Council of the College of Medicine /University of Babylon as a Partial Fulfillment for the Requirements for the Degree of Master in Science / Clinical Biochemistry

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Ministry of Higher Education
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دراسة بعض المؤشرات الحيوية في المصل والمجموعات الوظيفية في اللعاب في مرضى القصور الكلوي

رساله
مقدمه الى مجلس كليه الطب في جامعة بابل
وهي جزء من متطلبات نيل درجة الماجستير
في العلوم/ الكيمياء الحياتية السريرية

من قبل

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بسم الله الرحمن الرحيم

صدق الله العلي العظيم

سورة المجادلة (أية 11)

Supervisors Certification

We certify that this thesis entitled **(**Study of Some Serum Biomarkers and Salivary Functional Groups in Patients with Renal Impairment) has been prepared under our supervision at the Department of chemistry and Biochemistry, College of Medicine, University of Babylon, in partial requirements for the degree of master in Clinical Biochemistry.

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Date / / 2023

College of Medicine/ University of Babylon

Dedication

I dedicate this work to :

My Died Father.

And

My Husband for Supporting and Encouraging Me

My mother , My brother, My sister

And …… My (Son And Daughter)

Zahraa

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List of Abbreviation

Abbreviation	Description
AKI	Acute Kidney Injury
ARF	Acute Renal Failure
ATR-FTIR	Attenuated Total Reflection-Fourier Transform Infrared Radiation
AUC	Area Under the Curve
BTP	Beta Trace Protien
CKD	Chronic Kidney Disease
CVD	Cardio Vascular Disease
CNS	Central Nervous System
EPO	Erythropoietin
GFR	Glomerular Filtration Rate
GLUT	Glucose transport protein
KD	Kidney Disease
LFABP	Liver Fatty Acid Binding Protien
MDRD	Modified Diet in Renal Disease Equation
PKD	Polycystic Kidney Disease
SGLT	Sodium-dependent glucose transporters
WHO	World Health Organization
RAAS	Renin Angiotensin –Aldosterone System
DKA	Diabetic Ketoacidosis
HHS	Hyper Osmolar Hyperglycemic State
CRF	Chronic Renal Failure
NAGL	Neutrophil Gelatinase Associated Lipocalin
NAG	N acetyl B -D Glucosaminidase
KIM-1	Kidney Injury Molecule
ADMA	Asymmetric Dimethyl Arginine
PTX3	Pentraxin-3
UMOD	Uromodulin
TNF	Tumor Necrosis Factor

Summary

Kidney Disease (KD) is characterized by a progressive and irreversibly loss of kidney function or persisting renal damage, resulting from many underlying factors such as diabetes mellitus and hypertension. It can be classified based on the cause, severity of structural and functional abnormalities, and duration of those abnormalities. The current study was designed to evaluate the use of beta trace protein(BTP) and liver fatty acid binding protein (LFABP) as recent markers of renal disease and measured by using enzyme linked immunosorbent assay (ELISA). Also, to examine salivary components mainly (hydroxyl and amide) vibration bands as indicator for initiation of renal abnormality by using attenuated total reflection-fourier transform infrared (ATR-FTIR) spectroscopy.

Current study was included 107 subjects, (27) samples were excluded due to analytical defects especially for saliva samples. Remaining 80 participants with an age range between 25-63 years and with BMI 19-29 Kg/m^2. These participants were subdivided into 38 kidney disease patients (19 with acute kidney injury and 19 chronic kidney disease) and 42 apparently healthy volunteer. In present study were estimated serum levels of (glucose, urea, creatinine and albumin)by colorimetric methods, the total and ionized calcium by using electrolyte analyzer and also, investigated serum beta trace binding protein and liver fatty acid binding protein with salivary hydroxyl and amide vibration bands.

The results of present study revealed significantly ($p < 0.05$) increase of urea and creatinine in kidney disease patients when compared with control, While glomerular filtration rate (GFR), (total and ionized) calcium were a significantly (p value<0.05) decrease at the same comparison with a

significantly ($p < 0.05$) changes of glucose level. On other hand the beta trace binding protein increase significantly (p value<0.05) in chronic kidney disease subgroup in comparison with acute kidney injury and control groups mean difference were (21.974, 20.814) respectively. While liver fatty acid binding protein decrease significantly (p value<0.05) in acute kidney injury when compared with groups of chronic kidney disease patients and control (21.222, -15.306) respectively. Otherwise in chronic kidney disease the salivary hydroxyl and amide bands were significantly reduced in comparison with acute kidney injury patients and control group.

The correlation analysis among kidney disease patients shows positive correlation between beta trace and liver fatty acid binding protein (r=0.815, p=0.000), negative correlation was observed of glomeruli filtration rate with beta trace protein and liver fatty acid binding protein (r=0.414, P=0.010), (r=0.350, p=0.036) respectively. Furthermore positive correlation was recorded between salivary hydroxyl and amide bands (r=0.564, p=0.000). Outcome of ROC analysis was recorded very strong result of salivary hydroxyl band in chronic kidney disease patients than control (AUC) 91.1%, P =0.000, cut-off the test equal to (3373.20 cm^{-1}) . Likewise, (AUC) of salivary hydroxyl for patients chronic kidney disease than acute kidney injury was 83.41% , P =0.000 at the cutoff (3343 cm^{-1}).

In conclusion, the findings of this study suggest that liver fatty acid binding protein can be considered a potential marker for the progression of renal failure, while Beta trace protein showed less sensitivity and may require further investigation. Additionally, salivary hydroxyl bands were found to be highly sensitive indicators for the initiation of renal abnormality. The results highlight the potential utility of these markers in diagnosing and monitoring kidney disease.

1. Introduction

Kidney Disease (KD) characterized by a progressive and irreversibly loss of kidney function or persisting renal damage[1]. Reflects the entirety of acute kidney diseases and chronic kidney disease [2]. Acute insults to the kidney were typically termed “acute renal failure” and categorized according to the clinical presumption of the site of injury: “prerenal,” “renal,” and “post renal ”. Chronic kidney disease (CKD) and its terminal complication, end-stage renal disease, may progress undetected until immediately before symptomatic kidney failure develops [3], while if the renal failure is not diagnosed lead to permanent kidney damage fluid up, chest pain, muscle weakness, vomiting and Death[4].

The important causes of renal failure such as Diabetes Mellitus ,hypertension , Immune disease, hyperlipidemia ,smoking and kidney stones(are mineral deposits in the renal calyces and pelvis that are found free or attached to the renal papillae) [5].

In addition to protein components, saliva contains a large variety of low molecular weight substances, amino acids and hormones. The relationships between the functional state of the human body and the salivary gland’s physiological activity offer the possibility to use saliva as a source of diagnostic information, with a number of advantages over analysis of other biological fluids such as Breast cancer, cardiovascular disease and COVID 19 [6].

The recent discovered markers are beta trace binding protein(BTP) and liver fatty acid binding protein(L-FABP), BTP originating from kidneys, genital organs, heart ,cerebrospinal fluid and the liver, eliminates highest

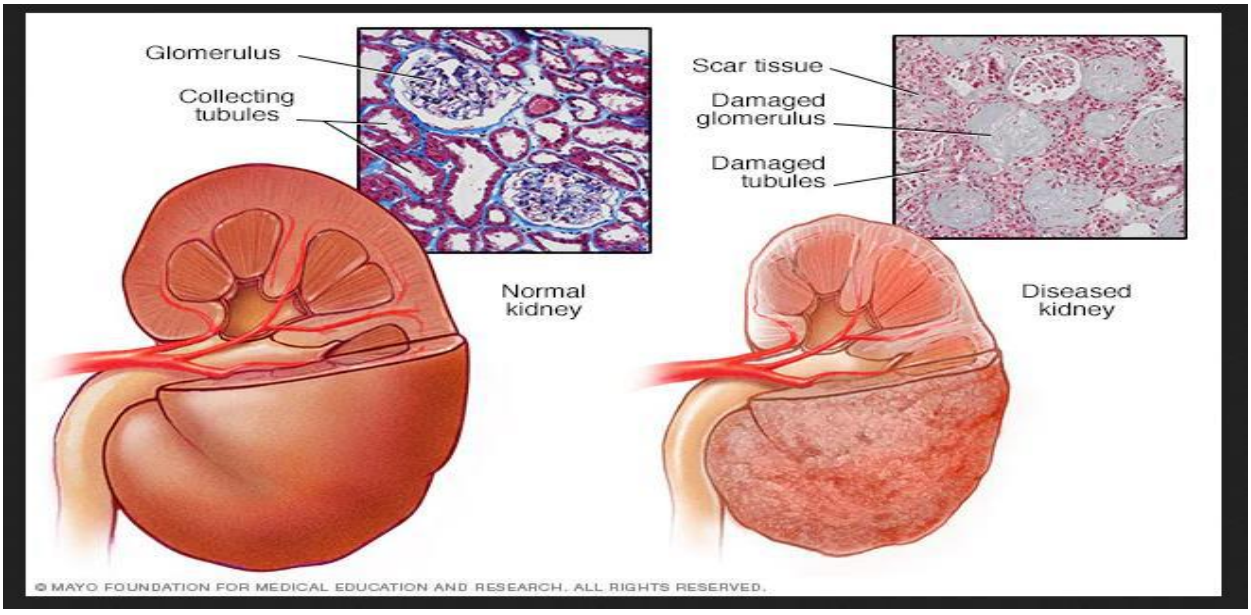
concentrations of BTP are observed in cerebrospinal fluid, making it a marker for distinguishing between cerebrospinal fluid leak and other bodily fluids[7]. The BTP molecules with smaller carbohydrate residues, reducing the molecular weight range of BTP in serum to 26–29 kDa. BTP is not physiologically inert. It possesses both ligand-binding and enzymatic properties. BTP catalyzes the conversion of prostaglandin H_2 (PGH_2) to PGD_2. PGD_2 is an eicosanoid involved in a variety of important physiologic processes, including platelet aggregation, vasodilation, inflammation, adipogenesis, and bone remodeling [8].

The second marker is liver-type fatty acid binding protein (L-FABP) is abundantly expressed in hepatocytes and in proximal renal tubular cells with molecular weight 14kDa. Injury of the proximal tubular cells induce upregulation of the L-FABP gene, leading to increased L-FABP expression by these cells and increase in the urinary L-FABP excretion , which binds to long-chain fatty acids (LCFAs) and certain other lipids, has been found in various tissues, including the mammalian intestinal mucosa, liver, myocardium, adipose tissue, kidney, muscle, and other tissues [9][10]. Recent study have shown that L-FABP may play an important role in kidney injury and repair, that monitoring detectable L-FABP levels can predict the occurrence and severity of various kidney diseases, and that this protein may be a promising biomarker of kidney disease[11]. The previous studied indicated the LFAPB and BTP are the emerging biomarker[12].

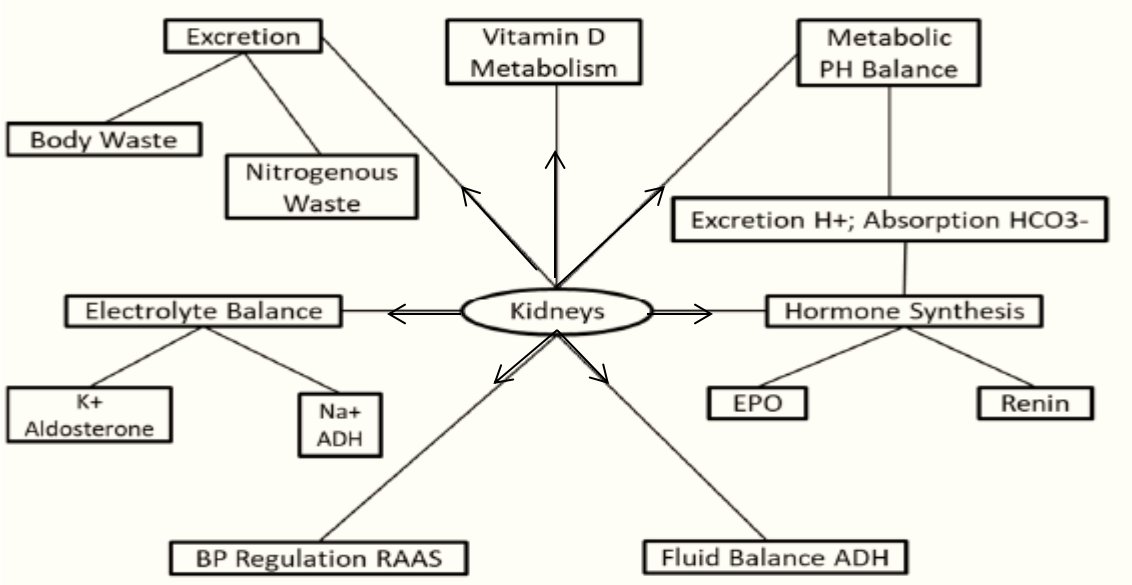
1.1 Kidney disease

1.1.1 Definition

Kidney disease is defined as the kidneys' inability to conduct excretory activities as in Figure(1-1), which results in the accumulation of nitrogenous wastes in the bloodstream. The kidneys have endocrine and exocrine functions regulating and maintaining critical biological mechanisms in the body. The exocrine function involve fluid and electrolyte balance, acid base regulation and excretion body waste. The endocrine functions include the activation of vitamin D for the incorporation of calcium into bones, and also synthesis of the blood pressure-regulating hormones renin-angiotensin and aldosterone, as well as the hormone erythropoietin, which is responsible for the production of red blood cells as in Figure (1-2) [13].



Figure(1-1) Normal kidney and kidney injury[14]



Figure(1-2) The Physiological Functions of Kidneys [15]

The AKI and CKD are common clinical diseases that result in renal failure and a series of clinical syndromes. The increasing incidence of these diseases is an important cause of increasing mortality worldwide. Identifying these patients early is of great importance both for intervening in a timely manner and for improving prognoses[9][15]. A decline in the GFR 30% or 40% is a step on the path to kidney failure [16] as in figure (1-3).

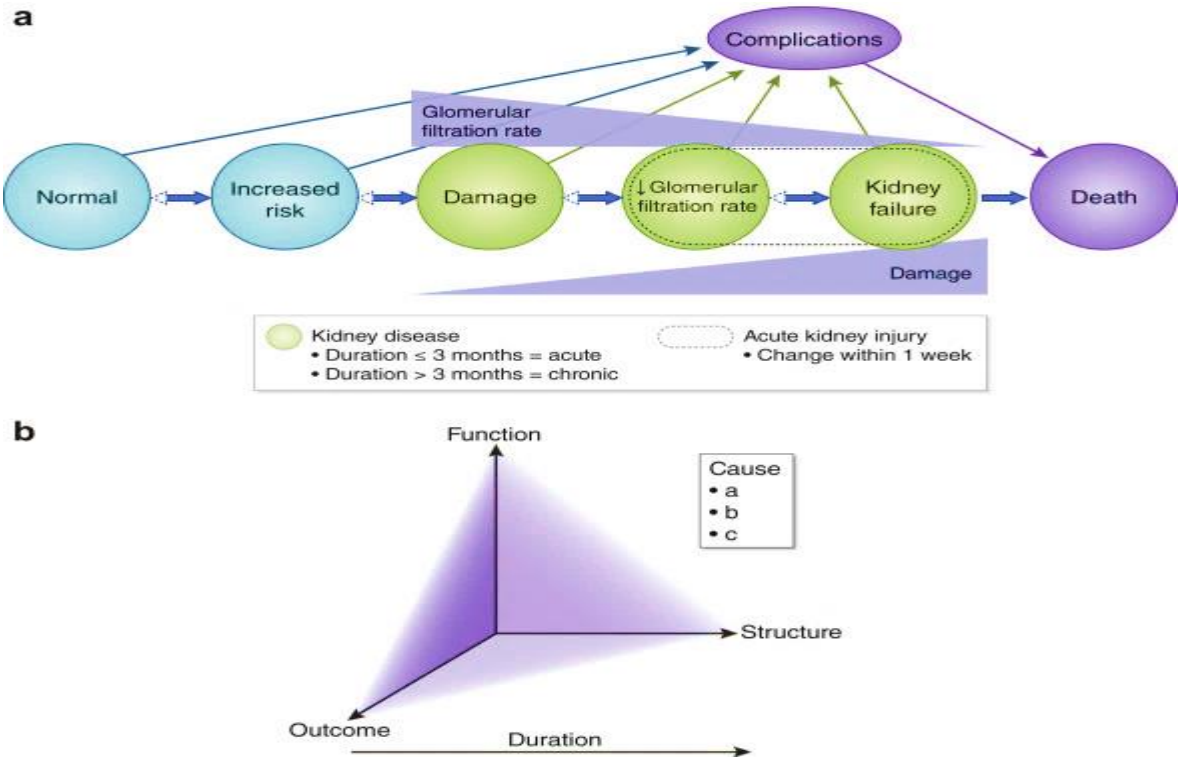


Figure (1-3) Conceptual model of Renal decline [17]

1.1.2Types of Kidney Disease

1.1.2.1Acute Kidney Injury(AKI)

Acute Kidney Injury (AKI) is the term that has recently replaced the term acute renal failure. AKI is defined as an sudden (within hours) decrease in kidney function, which includes both injury (structural damage) and impairment (loss of function). The diagnosis of acute kidney injury, and staging of its severity are based on changes in serum creatinine and urine output. It is a syndrome that rarely has a sole and distinct pathophysiology. Many patients with AKI have a mixed an etiology where the presence of sepsis, ischemia and nephrotoxicity often co-exist and complicate recognition and treatment. Furthermore the syndrome is quite common

among patients without critical illness and it is essential that health care professionals, particularly those without specialization in renal disorders, detect it easily[18][19]. Classification of AKI includes pre-renal AKI, acute post-renal obstructive nephropathy and intrinsic acute kidney diseases. Of these, only 'intrinsic' AKI represents true kidney disease, while pre-renal and post-renal AKI are the consequence of extra-renal diseases leading to the decreased glomerular filtration rate (GFR). If these pre- and/or post-renal conditions persist, they will eventually evolve to renal cellular damage and hence intrinsic renal disease. The risk of AKI in the elderly is high, due to the several anatomic and physiological changes of

the aging kidney [20]. The prostate processes (benign hypertrophy /carcinoma), retroperitoneal, adenopathy, malignancies, and neurogenic bladder are the most common causes of obstructive AKI in men, whereas pelvic and retroperitoneal carcinomas in the women. Nephrolithiasis and calculi are common causes of post-renal AKI in the elderly also the long-term complications of diabetes[20] . Diabetes also increases the risk of cardiovascular disease (CVD) and heart failure, and these conditions can themselves increase the risk of AKI as in figure (1-4). The higher risk for progression to chronic kidney disease (CKD), end-stage-renal disease (ESRD), and death [17].

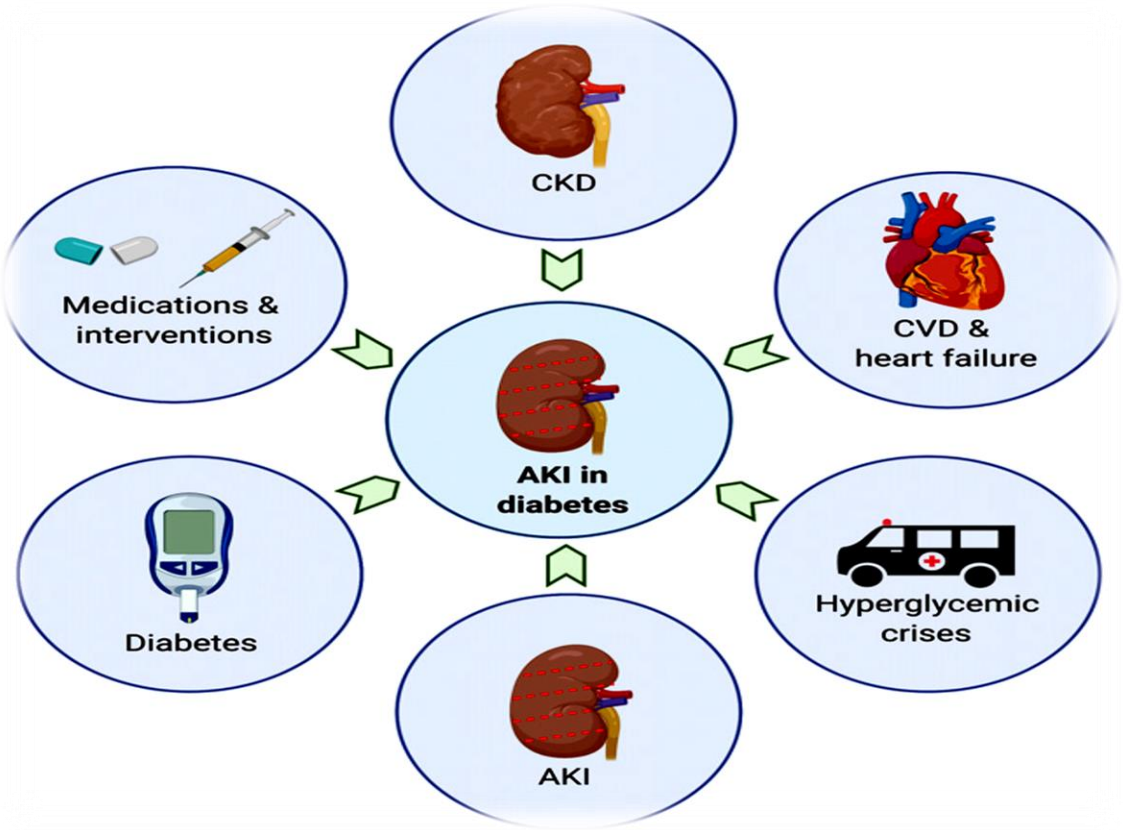


Figure (1-4) Causes of AKI in diabetes. Illustrating the major contributors to the increased risk of AKI in diabetes. These include CKD; CVD, including acute coronary artery syndromes and cardiac surgery; heart failure; hyperglycemic crises (DKA and HHS); AKI itself (predisposing to future episodes of AKI); diabetes itself (hyperglycemia); and sometimes medications used in the treatment of diabetes or its complications or in the investigation of comorbidities in persons with diabetes (e.g., radiocontrast) [22]

1.1.2.2 Chronic Kidney Disease(CKD)

The CKD is a general term for mixed disorders affecting the structure and function of the kidney (i.e., persistent urine abnormalities, structural abnormalities or impaired excretory renal function suggestive of a loss of functional nephrons). The Kidney Disease Outcomes Quality Initiative

(K/DOQI) of the National Kidney Foundation (NKF) have been defined CKD by either GFR < 60 ml/min/1.73 m² or the presence of kidney damage for more than 90 days duration via clinical assessment (Duration is necessary to distinguish chronic from acute kidney disease)[23]. The patient with CKD appear various structural and functional changes within the kidney will develop during the disease course, resulting in glomerular, tubular and vascular injuries, the progression phase of the disease is characterized by a persistent state of inflammation, oxidative stress and hypoxia as in figure(1-5) that contribute to the development of renal fibrosis [24], and in figure (1-6) shows the factor effecting of chronic kidney disease.

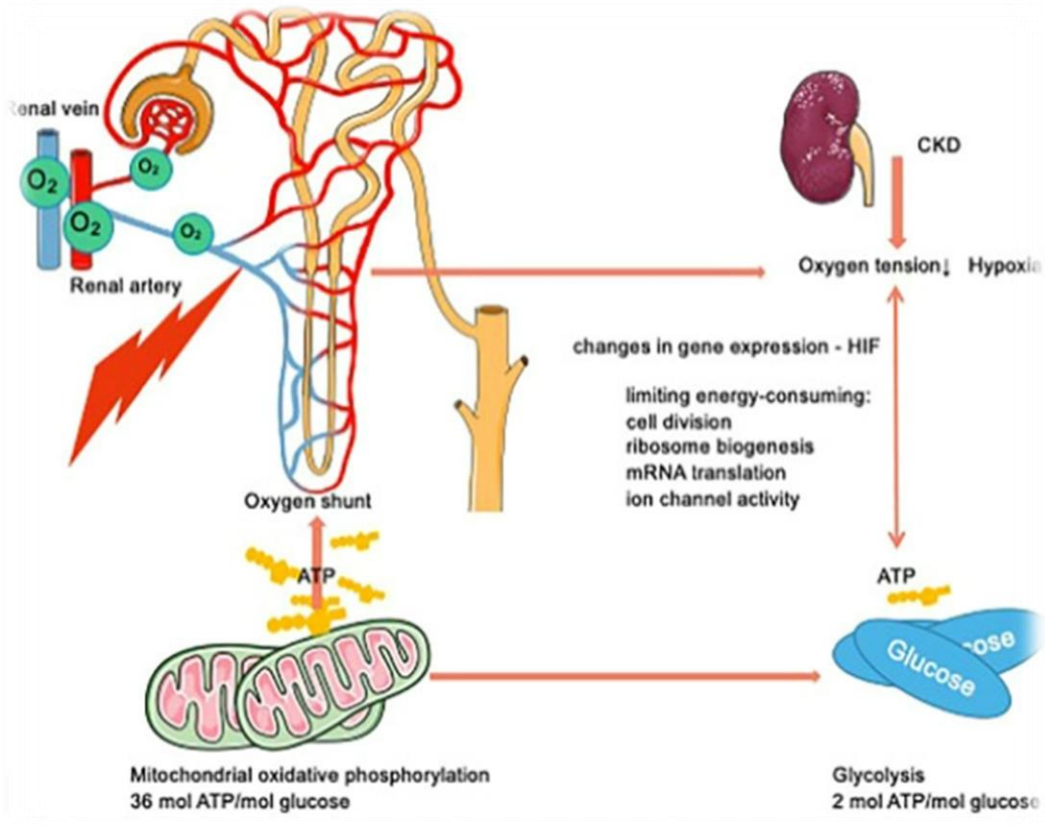


Figure (1-5) Hypoxia and Chronic Kidney Disease [25]

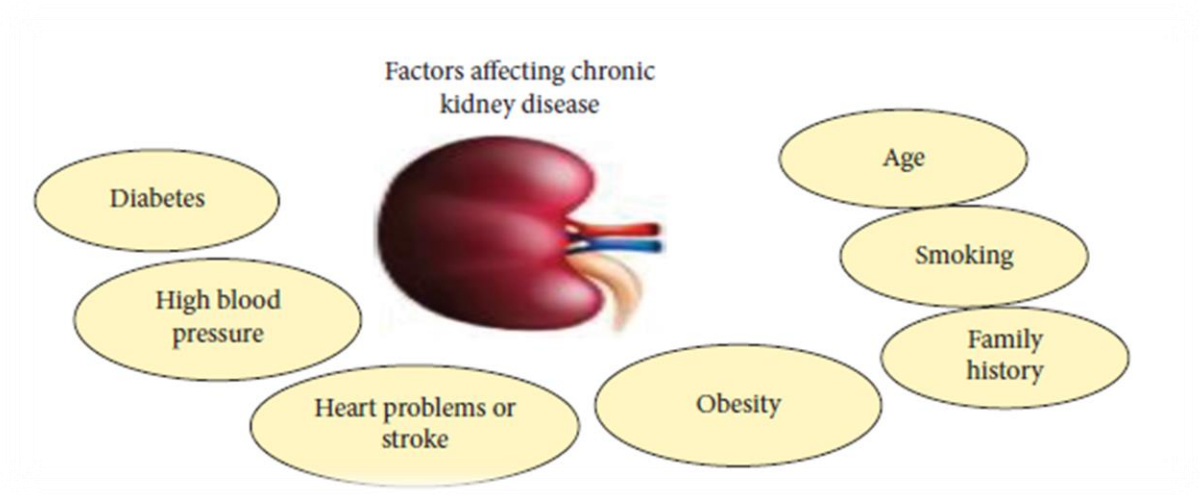


Figure (1-6) Factors affecting Chronic Kidney Disease [26]

1.1.3 Epidemiology

Studies from around the world reported an age-standardized global prevalence of CKD stages 1–5 in individuals aged ≥20 years were 10.4% among men and 11.8% among women. The study reported important differences by geographic region classified by income level, with a CKD age-standardized prevalence of 8.6% and 9.6% in men and women respectively.

In high-income countries, and 10.6% and 12.5% in men and women respectively in low- and middle-income countries. The age-standardized global prevalence of CKD stages 3–5 in adults aged ≥20 years in the same study was 4.7% in men and 5.8% in women [27]. The CKD affects 37 million adults in the USA .Recent studies suggest that disease-causing genetic variants are identifiable in ~10% of adults and ~20% of children

with CKD most of whom are unaware of the genetic etiology for their kidney dysfunction. CKD increased by 41.5% between 1990 and 2017 [28].

The prevalence of CKD stages 3–5 was varied among sub regions and country economic classification. CKD prevalence was 8.6% (7.2–10.2%) in east Asia, 12.0% (7.7–17.0%) in south-east Asia, 13.1% (8.7–18.2%) in western Asia, and 13.5% (9.5–18.0%) in south Asia. CKD prevalence was 9.8% (8.3–11.5%) in upper-middle-income countries and 13.8% (9.9–18.3%) in lower-middle-income countries [29].

Until December 30, 2017 the overall prevalence of chronic kidney disease Iranian general population in 70 605 people was 15.14% (I2 = 99.77%). The prevalence of CKD in female patients (18.80%) was 1.7 times higher than in male patients [30].

The CKD prevalence within this segment of the Saudi population at 5.7% using the modification of diet in renal disease (MDRD) equation and 5.3% using the chronic kidney Disease epidemiology collaboration(CKD-EPI) equation [31].

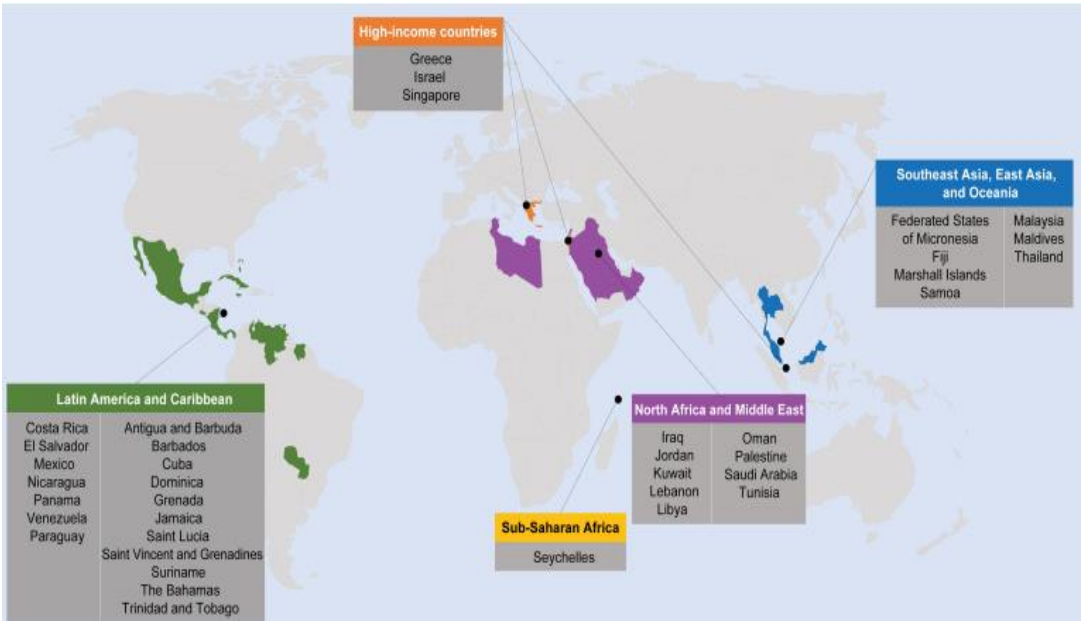


Figure (1-7)Regions and countries where Chronic Kidney Disease is in the top 10 causes of years of life lost in 2013. On the basis of data from the Global Burden of Disease Study 2013 [27]

1.1.4 Etiology and Risk Factor

Kidney Disease (KD) incidence and progression are commonly believed to be based upon group differences in the prevalence of risk factors for KD , such a :

1. Diabetes: the diabetes can harm the kidney by causing damage . The high glucose level in blood can cause these vessels of the kidney to become narrow and clogged without enough blood ,the kidney become damage and the protein (albumin) passes through the filter and end to the urine .Also, the diabetes damage the nerves in your body including bladder and if the urine stay for a long time in bladder may get a urinary tract infection[32] .

2. Emphysematous pyelonephritis: elevated blood and tissue glucose levels may contribute to a conducive environment for the rapid multiplication of

gas forming bacteria. Diabetic microangiopathy causes a slowing down of transport and drainage of the catabolic waste products from the kidney, resulting in stasis or accumulation of such products within the renal parenchyma. This causes the accumulation of gas within the kidney and results in further deterioration [32].

3. Kidney Stones (Nephrolithiasis): The most common stone type is calcium oxalate (67%) followed by calcium phosphate (17%), uric acid (8%), struvite (3%), and cysteine (0.4%). Urinary stone formation is a multifactorial process to which metabolic derangements[33]. Nephrolithiasis can cause post-renal acute Kidney Injury (AKI) via obstruction of urinary outflow, often associated with rapid deterioration in renal function. Irreversible kidney damage can result if urinary drainage is not corrected in a timely fashion. Recent studies also suggest that patients with nephrolithiasis are more likely to develop Chronic Kidney Disease (CKD) [34].

4. Heavy Metals: The most common metals implicated in kidney toxicity are arsenic, cadmium, cobalt, lead, lithium, and mercury[35]. The Kidney is a target organ in heavy metal toxicity for its capability to reabsorb and concentrate divalent ions and metals. The magnitude of kidney impairment depends on the nature, the dose, and the time of exposure. These compounds are s reabsorbed through an endocytotic process in segment S1 of the proximal tubule and can lead to chronic inflammation, fibrosis and renal failure [36].

5. Polycystic Kidney Disease (PKD) : is a widespread hereditary cause of child and adult chronic renal failure is a diverse class of disorders characterized by fluid-filled cyst aggregation, development and growth in kidney and other organs sometimes induced end stage renal failure. As

autosomal dominant or autosomal recessive traits, the most frequently inherited PKD can be transmitted. The definition of PKDs has been limited by tradition to two conditions: autosomal dominant PKDs and autosomal recessive PKD. Collecting tubules are generalized in recessive polycystic renal disease, while cysts form in the nephron in prevalent polycystic disease[37] [38].

1.1.5 Pathophysiology

Pathological events can be induced by extrinsic events such as hypertension, obesity, sepsis, liver failure and diabetes[39]. Involve changes in the structure of the functional unit of the kidney called the nephron. This includes changes to the tubules, glomeruli, the interstitial and the intra-renal blood vessels. Kidney filtering waste from the blood, maintaining the overall fluid balance of the body, maintaining blood pH, and hormonal functions that promote red blood cell production, bone health and regulation of blood pressure[40]. Loss of enough cells along any part of the nephron can alter any of these functions. This is particularly true of the proximal tubules, which are the primary target of a vast majority of nephrotoxicants. The mechanisms mediating renal cell death induced by nephrotoxicants and renal pathologies. For example, ischemia-induced AKI involves ATP depletion, oxidative stress, proximal tubule cell death and loss of the brush border membrane, and cell polarity [41]. In comparison, AKI induced by the cancer chemotherapeutic and prominent nephrotoxicant cisplatin also involves oxidative stress, proximal tubule cell death and loss of the brush border membrane and polarity increased oxidative stress, loss of ATP and proximal tubule cell death are also commonly seen in nephrotoxicity induced by contrast media, which is also known to alter

glomerular function and renal blood flow[42]. Diabetic induced nephropathy, proteinuria, glomerular fibrosis and interstitial fibrosis are key events in renal failure induced by hypertension and hyperglycemia [43].

Renal stone condition is widespread in a working-age demographic and has a considerable health care burden. The latest literature suggests that influences in lifestyle and diet play a significant role in stone disease risk. Genetic, nutritional, and environmental factors can influence the occurrence and prevalence of kidney stones. About 80 percent, typically calcium oxalate and less so often calcium phosphate, of the stones are composed of calcium salts in most industrialized nations[44]. The other 20 percent of stones are uric acid, struvite or apatite carbonate, cysteine, and rare stones. An elevated risk of renal stone is associated with a family history of kidney stones, insulin-resistant states, a history of hypertension, primary hyperparathyroidism, a of gout, chronic metabolic acidosis, and surgical menopause. The development of kidney stones in postmenopausal women is related to a history of hypertension and a poor diet of magnesium and calcium[45].

1.1.6 Complication of Kidney Disease [46]

1. Hypertension
2. Cardiovascular complication
3. CKD-related mineral bone disorder
4. Anemia
5. Salt and water retention
6. Metabolic acidosis and electrolyte disorders

1.2 Saliva and Infra-Red Spectroscopy:

The rapid advancements in technology have paved the way for novel diagnostic approaches that were not available in the past. One such example is the utilization of spectral techniques in clinical diagnosis. This includes various techniques such as nuclear magnetic resonance spectroscopy, nuclear magnetic imaging, mass spectrometry, atomic absorption spectrometry, and infrared spectroscopy, as referenced in sources. These techniques have proven to be valuable tools for accurate and efficient diagnosis of various diseases, and can be particularly useful in analyzing diagnostic fluids [47][48]. Research has revealed that several other bodily fluids, such as saliva, cerebrospinal fluid, and pleural fluid, may contain a wealth of biological information that exceeds that of blood. These fluids may contain a range of reliable disease markers that can be leveraged to diagnose a variety of illnesses. Therefore, these alternative fluids represent promising areas for further investigation and development of diagnostic techniques [49][50].

One of the most common diagnostic fluids is saliva which is a multifaceted biological fluid that contains a diverse array of biomolecules including DNA, RNA, proteins, metabolites, and microbiota. These biomolecules make it a valuable tool for the detection and diagnosis of various diseases. Moreover, its simple collection process, non-invasive nature, and easy storage make it a potential substitute for blood in the realm of fluid biopsy and an excellent candidate for use as a diagnostic fluid [51].

Izabella C. C. Ferreira and her team utilized ATR-FTIR to analyze the saliva of patients diagnosed with breast cancer, benign breast disease, and healthy matched controls in order to explore its potential as a diagnostic

tool for breast cancer. Through this process, the team identified several vibrational modes in the original and second-derivative spectra of the saliva samples . The team's analysis revealed that there were notably higher absorbance levels at the 1041 cm^{-1} wavenumber in the saliva samples of breast cancer patients as compared to those of benign patients (with a p-value <0.05) figure (1-8) [52] [53].

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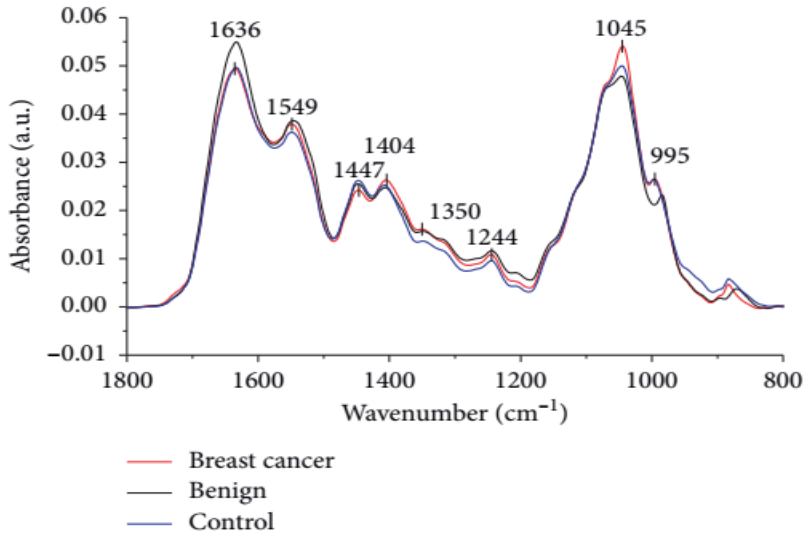


Figure (1-8) : displays the FTIR spectra of the saliva samples collected from breast cancer, benign breast disease, and control patients. The graph showcases the average original spectra for each group, with the absorbance bands of the major functional groups in biomolecules indicated between wavenumbers 1800 cm^{-1} and 800 cm^{-1}. The spectra for breast cancer patients are represented by the red line, those for benign breast disease patients by the black line, and the spectra for control saliva samples are denoted by the blue line[53]**.**

As previously stated, the use of saliva presents a promising new avenue for fluid-based diagnostics. As salivomics research continues to accumulate data, it is anticipated that a group of salivary biomarkers will be identified for a wide range of diseases. This development will help enable more common and accurate diagnoses using saliva samples.

1.3 Renal Function Test

1.3 .1 Urea

Urea is a small water-soluble molecule that is freely filtered by the glomeruli and absorbed by the proximal and distal tubules of the kidney. Several urea transporters are involved in urea handling along the nephron. Urea has quantitively the highest serum concentration among the different organic solutes retained in patients with CKD[54].

Blood urea nitrogen (BUN) is one of the indicators for evaluating kidney function, and BUN levels are inversely correlated with kidney function. Besides glomerular filtration, BUN levels are also influenced by tubular resorption and production of urea. In acute illnesses, such as acute heart failure, acute pancreatitis and extensive diuretic use, increased tubular reabsorption of urea consequent to neuro humoral activation originating from the depletion of effective circulating volume is a common cause of higher elevation of BUN compared to glomerular filtration rate (GFR) leading to higher BUN/creatinine ratio in these patients[55].

1.3.2 Creatinine

Creatinine is synthesized in liver, kidney and pancreas , transported to the sites of usage, principally muscle and brain, it is generated from the breakdown of muscle creatine phosphate, and is excreted in the urine by means of passive glomerular filtration with subsequent minimal tubular reabsorption and secretion. The generation of creatinine is quite stable and as long as (GFR) remains unchanged, a steady-state of its generation and excretion is achieved, reflected by stable levels of serum creatinine (SCr)[56]. Thus, SCr is a useful endogenous indicator of GFR, as long as its

generation and elimination remain stable. When GFR declines, SCr increases. Where the continuous daily excretion of creatinine equals to its generation.

Estimated GFR can be calculated by urine collection and concomitant determination in plasma and urine of endogenous biomarkers, such as creatinine, or of exogenously administered compounds such as inulin [57].Thus, creatinine clearance roughly represents the mean daily GFR. Several widely used formulas provide an estimate of GFR (eGFR), based on SCr and on anticipated muscle mass (governed by age, gender, weight and race), enabling a close estimate of GFR without the need of urine collection and some medications that inhibit tubular excretion of creatinine, such as cimetidine or trimethoprim [58].

1.3.3 Glomerular Filtration Rate (GFR)

Kidney disease is common in adults, and testing for kidney disease is part of routine clinical practice for patients with acute or chronic illness. The initial evaluation includes determination of the glomerular filtration rate (GFR), estimated on the basis of the serum creatinine level (e GFR cr). Assessment of GFR is central to clinical practice, research and public health. In clinical practice the GFR is used to interpret the symptoms, signs, and laboratory abnormalities that might signify kidney disease; to adjust drug doses; and to detect, assess risk, and manage acute kidney diseases with disorders(AKD) and CKD[59].

1.3.4 Albumin

The increased excretion of urinary albumin as a marker of kidney damage. Normal individuals excrete very small amounts of protein in the

urine. Albumin is the most common type of protein in the urine. All patients with CKD should be screened for albuminuria. Persistent increased protein in the urine (two positive tests over 3 or more months) is the principal marker of kidney damage, acting as an early and sensitive marker in many types of kidney disease [60][61].

1.3.5 Glycosuria

Abnormally high levels of glucose in blood (hyperglycemia) can lead to glycosuria. Renal glycosuria occurs when the renal tubules fail to reabsorb all glucose at a level that is normal. <140mg/dL is ideal. Over 200mg/dl can indicate diabetes. A glucose in urine test measures the amount of glucose in the urine, the blood carries glucose to cells. The CKD does not cause diabetes, but CKD can be a complication of diabetes [62][63].

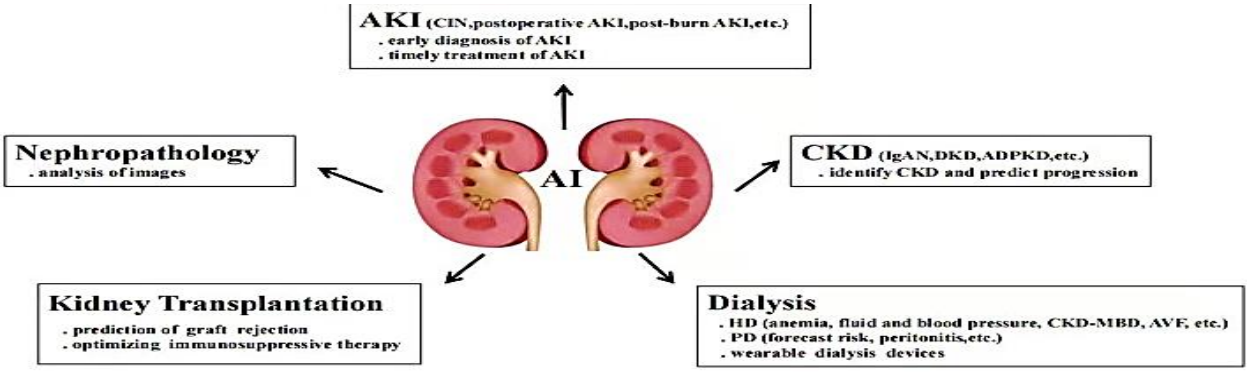
1.3.6 Calcium

Calcium balance is regulated by intestinal calcium absorption, kidney reabsorption and calciotropic hormones that activate calcium exchange from the bone when serum calcium levels are low. Research shows that ingesting about 800–1000 mg/day of calcium may be sufficient to maintain calcium balance for patients with CKD 3–4 in the absence of vitamin D analogues. However, calcium recommendations for early stages of CKD typically follow the RDA (1000–1200 mg/dl) for adults because the level of kidney function has not yet disrupted calcium balance, Calcium phosphate particles, but not phosphate, damage renal tubular cell[64].

1.4 Treatment of Kidney Disease

Treatment of Kidney Diseases (KD) can slow its progression to end-stage renal disease (ESRD). However, the therapies remain limited. Blood pressure control using angiotensin-converting enzyme (ACE) inhibitors or angiotensin II receptor blockers (ARBs) has the greatest weight of evidence and reduced albumin in urine. Glycemic control in diabetes seems likely to retard progression. A sodium-glucose cotransporter (SGLT 2) inhibitor on initial changes in estimated glomerular filtration rate (e GFR) and describe that with the initial dip in e GFR, there is an associated improvement in kidney function[65]. Also used Diuretics are frequently prescribed drugs and help managing several pathological conditions, including acute and chronic kidney disease, nephrotic syndrome [66]. At the end stages Dialysis and kidney transplant are the two treatments for kidney failure. The dialysis treatments or transplanted kidneys will take over some of the work of your damaged kidneys and remove wastes and extra fluid from your body. This will make many of your symptoms better [67]. Many patients with kidney failure are candidates for one of two dialysis approaches: hemodialysis (HD), typically provided in a hospital or other specialist facility (i.e., in-center), or peritoneal dialysis (PD), usually self-administered at home after training. It' s removing waste, salt and extra water to prevent them from building up in the body, keeping a safe level of certain chemicals in your blood, such as potassium, sodium and bicarbonate helping to control blood pressure[68]. Kidney transplantation is the best treatment method among all of the renal replacement therapy options (HD、PD and transplantation). However, few patients with ESRD benefit from kidney transplantation because of the limited number of organ donors. To optimize the allocation

of organs, complex -health systems have been proposed based on data mining and NN algorithms. The application of AI in kidney transplantation can be summarized from two aspects: prediction of graft rejection and augmentation of post-transplantation immunosuppressive therapy[69]as in figure (1-9).



Figure(1-9) Application of artificial intelligence in Renal Disease [69]

1.5 Biomarkers of Kidney Function

The search for new biomarkers should focus on better indicators of renal dysfunction than GFR and on markers of specific types of kidney injury, assessed in serum and/or urine. The study of specific biomarkers would allow the identification of kidney damage, and they should reflect the underlying pathophysiological processes of kidney damage, namely, changes in renal function, tubulointerstitial damage, endothelial dysfunction and inflammation, and/or cardiovascular risk. Kidney biomarkers can be classified according to the morph physiological characteristics of the nephron, related both to the renal function (glomerular and tubular) and to the integrity of its endothelial or epithelial cells as in figure (1-10) [70] .

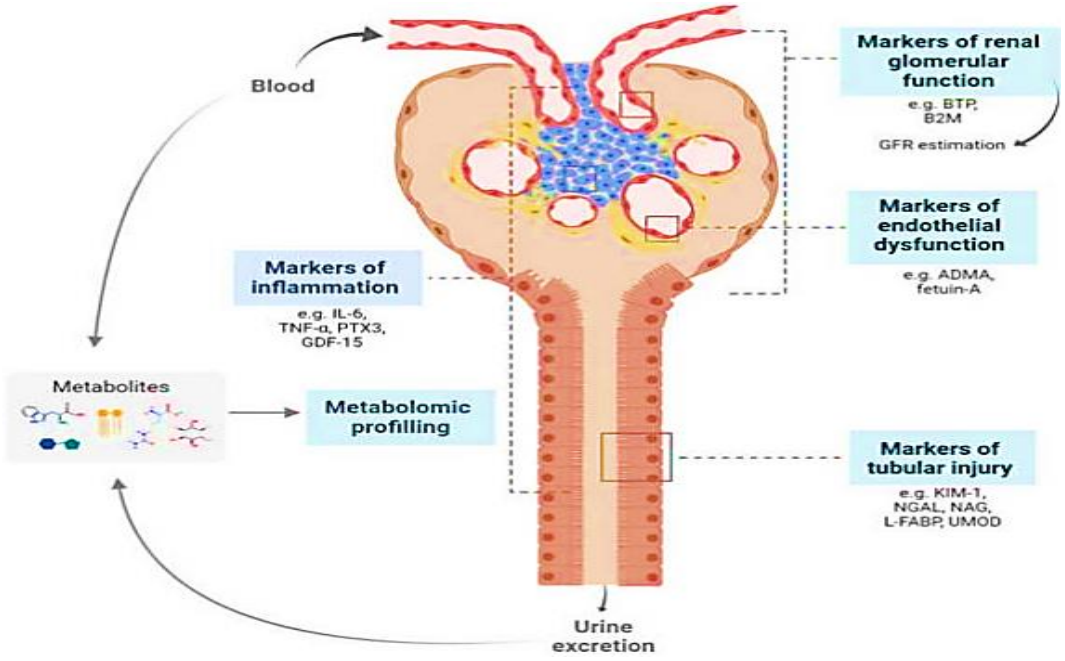


Figure (1-10) Biomarkers of Chronic Kidney Disease according to the anatomic localization and/or site of production [70]

1.6 Beta Trace Binding Protein

1.6.1 Beta Trace Protein Structure

Its micro heterogeneity is the consequence of post-translational N-glycosylation resulting in different glycoforms of varying molecular weight (23-29 kDa) encoded on chromosome 9, BTP is a heterogeneous monomeric glycoprotein with 168 amino acids as figure (1.11) [71]. These glycoforms are found in differing relative amounts in different fluid compartments. BTP was first isolated from cerebrospinal fluid and has been used as a marker of cerebrospinal fluid leakage, because it represents approximately 3% of total cerebrospinal fluid protein. More recently, BTP has been found to be expressed in the brain, retina, melanocytes, male genital organs, heart and kidney and it is secreted into various body fluids,

such as cerebrospinal fluid, seminal fluid, plasma and urine[72] . The larger glycoforms predominate in serum and urine and have more fully sialylated oligosaccharide chains [71]. The BTP is almost completely excreted by the kidneys. It is freely filtered by the glomeruli, reabsorbed and metabolized in the proximal tubule, with minimal tubular secretion, BTP is not completely reabsorbed by tubular cells and is detectable in urine of healthy individuals BTP was recovered in the urine, suggesting that BTP was primarily degraded in the tissues rapid elimination of BTP from the serum, with a half-life of <1 hour[73][74]. Previous studies documented the BTP's strong association with glomerular filtration rate, end-stage renal disease [75]. Since its urinary excretion is residual, the increase in these proteins has been proposed as potential markers of decreased GFR, and also as markers of tubular damage. serum BTP levels were found to be elevated in patients with decreased kidney function suggesting that this protein could be used as a diagnostic biomarker in early stages of kidney disease [76] .

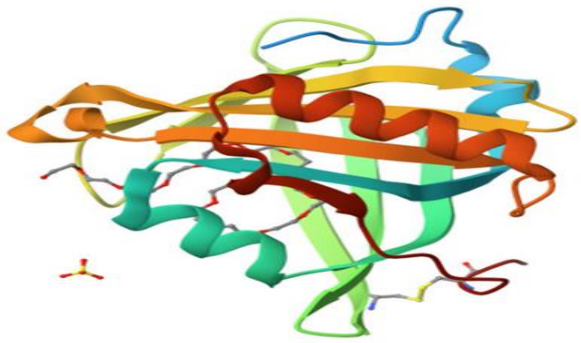


Figure (1-11) Three dimensional structure of Human lipocalin-type Prostaglandin D Synthase (BTP) from protein Data Bank[71] **.**

1.6.2 Beta Trace Protein Function

BTP is a member of the lipocalin superfamily, which consists of a group of secretory proteins that bind and transport lipophilic molecules. BTP binds with high affinity to a variety of lipophilic substances such as retinoid, thyroid hormones, bilirubin and amyloid B[77]. BTP also possesses enzymatic activities ,it is catalyzes the conversion of arachidonic acid derivative prostaglandin H2 (PGH2) to PGD2 [78] the mechanism seems in figure (1-12) .PGD2 is involved in a wide range of physiologic functions such as sleep induction and regulation, nociception, bronchoconstriction, adipocyte differentiation, nitric oxide release and induction of vasodilation, inflammatory mediator modulation, inhibition of platelet aggregation, skin homeostasis, bone remodeling, hair growth inhibition and immune system modulation in inflammatory conditions of the gastrointestinal tract, the function is seems as in figure (1-13) [79][80].

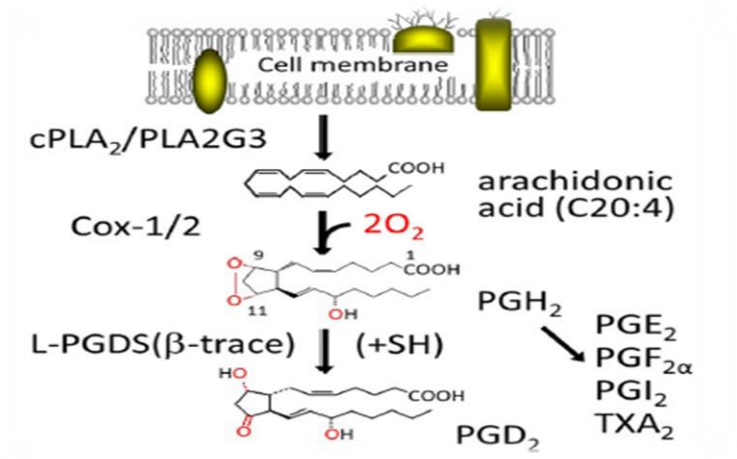


Figure (1-12) The biosynthesis of PGD2[78]

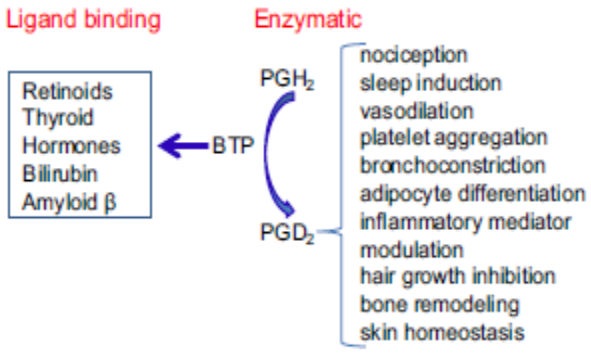


Figure (1-13) Dual functions of b-trace protein (BTP). BTP binds various lipophilic molecules and catalyzes the conversion of prostaglandin H2 (PGH2) to PGD2. PGD2 has a variety of physiologic function[81]

1.6.3 Location of Beta Trace Protein

BTP has been identified in many human fluids and tissues. BTP localizes to the leptomeninges, arachnoid cells, choroid plexus epithelial cells, and oligodendrocytes of the central nervous system (CNS). Certain cochlear and ocular cells, testicular Sertoli and Leydig cells, epithelial cells of the epididymis and prostate also contain BTP. In the heart, BTP has been localized to myocardial cells, atrial endocardial cells, synthetic state intimal smooth muscle cells and fibrous plaques of atherosclerotic stenosis coronary arteries (but not within normal coronary arteries). BTP has been described in vascular endothelial cells, skin melanocytes and keratinocytes, gastric mucosal epithelial cells, bone osteoblasts, and adipocytes. BTP localizes to cells of the proximal tubules, loop of Henle, and glomerulus. In most reports, BTP protein expression is accompanied by evidence that its messenger RNA (mRNA) is present, suggesting local BTP cell production[81].

1.6.4 Effect of Medication on Beta Trace Protein

Very little is known about the effect of medications on BTP. Immunosuppressive medications (low-dose prednisone, mycophenolate mofetil, cyclosporine, or tacrolimus) affected serum BTP. The lack of impact of low-dose steroids on serum BTP levels was reported in transplant cohort. A dose dependent reduction in serum BTP levels with relatively high doses of steroids has been described in pediatric patients after accounting for GFR[82]. Also glucocorticoid exposure significantly increases BTP expression in cardiomyocytes. The administrated of Estrogen is reported to increase myocyte BTP expression when the serum levels were not provided[83].

1 .7 Liver Type Fatty Acid Binding Protein (L-FABP)

1.7.1 Structure of Liver Fatty Acid Binding Protein

Mammalian intracellular FABP is a 14-kDa protein encoded by a large multigene family and is a member of the superfamily of lipid-binding proteins (LBP) with 126–137 amino acid residues [9] as in figure(1-14). Their tertiary structure resembles a clam shell in which the ligand is bound between the two halves of the clam by interaction with specific amino acid residues within the binding pocket, the so-called β-barrel , the primary function of FABP is the facilitation of intracellular long-chain fatty acid transport. A stable intracellular half-life of 2–3 days , a significant impairment of renal function leads to an increased half-life of L-FABP in the circulation[84]. The genes encoding FABP consist of four exons and three introns with tissue-specific expression. endogenous antioxidant protein from the lipid-binding protein superfamily is expressed in proximal tubular epithelial cells[85].

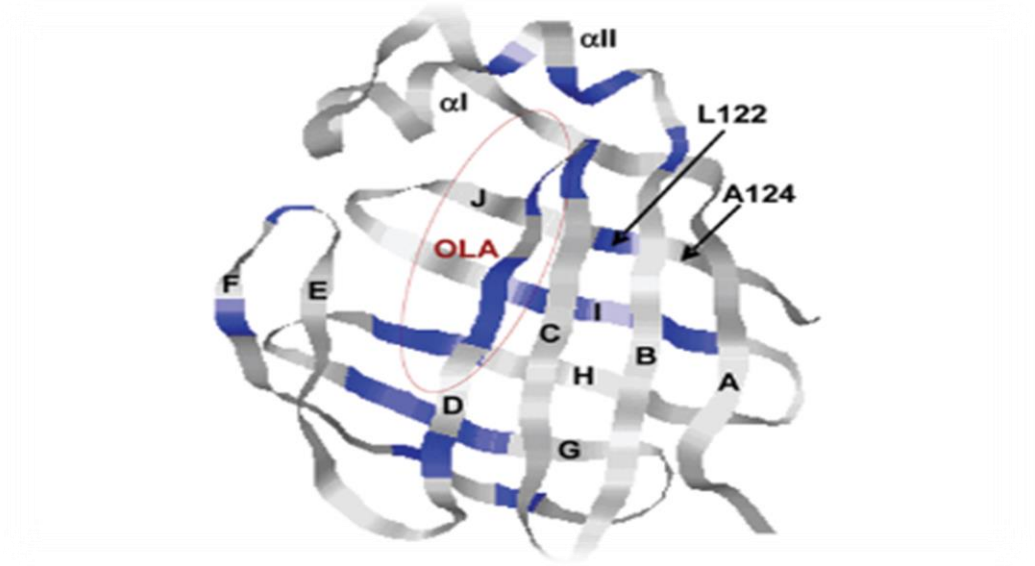


Figure (1-14) structure of LFABP[86]

1.7.2 Function of Liver Fatty Acid Binding Protein

Under physiological conditions, a percent of albumin is filtered from the glomeruli and reabsorbed predominantly in the proximal tubules bound to free fatty acids[87]. After reabsorption, cytosolic albumin releases fatty acids to L-FABP and moves into lysosomes during this process L-FABP binds long-chain fatty acids which plays a role in the fatty acid metabolism, intracellular signaling and promotes the excretion of lipid peroxidation products. In CKD patients, fatty acids overload the proximal tubule, and massive proteinuria is found. L-FABP expression and urinary excretion are increased by various stressors, such as proteinuria, hyperglycemia, tubular ischemia, toxins and salt-sensitive hypertension [88]. Urinary L-FABP levels accurately reflect the degree of tubular interstitial damage and are significantly correlated with the prognosis and progression of CKD. In diabetic patients, regardless of type, urinary L-FABP levels are higher in patients with norm albuminuria than in those

with microalbuminuria, thus, reflecting early stages of diabetic nephropathy [89].

1.7.3 Location of Liver Fatty Acid Binding Protein

Nine FABPs have been identified with tissue-specific distributions: L (liver), I (intestinal), H (muscle and heart), A (adipocyte), E (epidermal), B (brain), M (myelin) and T (testis) [9].

1.7.4 Effect Medication on Liver Fatty Acid Binding Protein

L-FABP are not influenced by its serum levels because urinary L-FABP originates mainly from the tubular cells. This biomarker is notes elevated in the early stages of diabetes and also influenced by lipid-lowering medication and angiotensin II receptor antagonists[90].

Aims of Study :

- Evaluation of beta trace protein , liver fatty acid binding protein as a recent markers of renal disease .
- Estimation the renal function tests and study the relationships with above markers for detect the progression of renal disease.
- Evaluation salivary hydroxyl and amide of total protein components as indicator for initiation of renal abnormality by using attenuated total reflection-fourier transform infrared(ATR-FTIR) spectroscopy.

2.1 Subjects:

This study was carried on patients attended to dialysis center in Imam Sadiq teaching hospital and AL Hilla teaching hospital in addition to control individual that included relative & medical staff in Hilla province that which apparently healthy. All samples were collected from 1st of September 2022 until 1st of December 2022. The experimental part of the study was performed at the laboratory of chemistry and biochemistry department in College of Medicine / University of Babylon.

2.1.1 Study Design: Case control study.

2.1.2 Study Population

The current study was included 107 subjects, (27) samples were excluded due to analytical defects especially for saliva sample. Remaining (80) subjects categorized in to three groups, the range of age and BMI were (25-63years), and (19 -29) respectively.

2.1.3 Study Groups:

1. **Acute Kidney Injury (AKI) Group:**

The patients (n=19) included (Male=15,Female=4).

2. **Chronic Kidney Disease(CKD) Group:**

The patients with CKD (n=19) included (Male=14,Female=5).

3. **Control Group:**

Included (n=42)(Male=30,Female=12)and these individuals who apparently healthy .These groups illustrated in figure (2-1) .

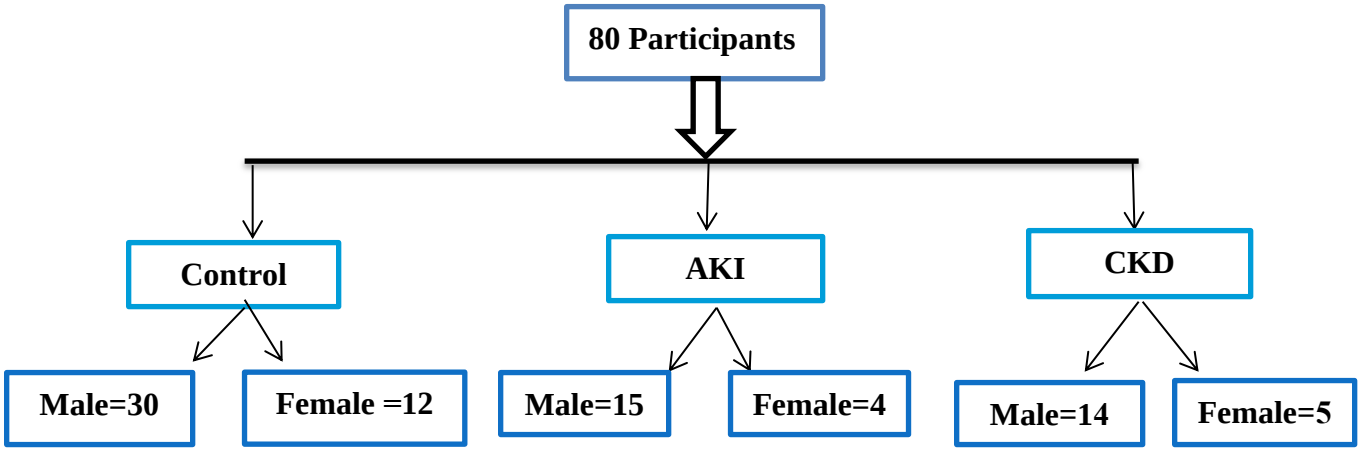


Figure (2-1): Simplified chart represents the study groups

2.1.4 Ethical Approval

The ethical approval depends on the following**:**

1. Approval of scientific committee of Babylon Medical College (University of Babylon, Iraq) and the Biochemistry Department at in the same college.
2. Approval of scientific committee of Imam Sadiq Teaching Hospital and Al Hila Teaching hospital/ Babylon city according to the document number 4 in 06/07/2022 to get this approval.

3. The objectives and methodology of this study were explained to all participants in the current study to gain their verbal acceptant.

2.1.5 Data Collection

For this study, the inclusion and exclusion criteria are as follows:

1. Inclusion Criteria:

- Patients with kidney impairment.
- Age between 20-65.
- BMI between 19-29.

2. Exclusion Criteria: liver disease , heart , thyroid and auto immune diseases in addition to obesity , pregnancy and smoking .

2.1.6 Samples Collection for Blood and Saliva :

Blood samples (5 ml) were aspirated without tourniquet from patients with kidney disease and control by using disposable syringe. Blood push slowly in gel tube and let to clot at room temperature for (10-15)min, then centrifuged at 3000 xg for 10 minutes. The serum was obtained and put in Eppendorf tubes (labeled) with number of sample then stored at -20°C until the time of examination and a pharyngeal swab was collected from participants by inserting a cotton swab into the mouth and scrapping the tonsils, the tongue, and the inner part of the cheek. The swab was then placed immediately into a sterile tube then labeled with number of sample and stored on deep freeze until analysis. Saliva sample was used to determine general content of hydroxyl band of carboxyl group and amide bands to examine the differences in studied groups.

2.2 Materials

2.2.1 Chemicals:

All chemicals and standard kits used in this study with their sources listed in the Table (2-1) :

Table (2-1): Chemicals substances and standard kits

No	Chemicals and Kits	Origin
1	Albumin kit	Biolabo (France)
2	Beta trace protein ELISA kit	China-BT(bioassay Technology Laboratory)
3	Creatinine kit	Biomaghreb (Spain)
4	Cleaning solution	China(Genrui)
5	De protein solution	China(Genrui)
6	Electrolyte reagent DS-l (standard A+ standard B)	China (Genrui)
7	Ethanol	Iraq (Tiebah)
8	Glucose kit	Biolabo (France)
9	Liver fatty acid protein ELISA kit	China-BT(bioassay Technology Laboratory)
10	Urea kit	Biolabo (France)

2.2.2 Instruments and Equipment

The instrument and equipment's and sources used in this research were listed in Table (2-2) .

Table (2-2) Instruments and equipment:

No	Instruments	Origin
1	Blue and yellow tips	China
2	Deep freeze	GFL/Germany
3	Disposable Syringes	Emirate
4	Distiliter	GFL/Germany
5	ELISA (reader-washer-printer)	Biotic/ USA
6	Eppendrof tube(1.5)	China
7	Filter papers	China
8	FTIR spectrometer with ATIR unit	(Bruker) Tensor27
9	Gel tube	Jordon
10	Incubator	Fisher cient/Germany
11	Macro Centrifuge	China
12	Micropipettes(5-50micron)	Germany
13	Multichannel micropipette	Germany
14	Saliva (swab)	Swab (china)
15	Spectrophotometer CECIL 7200	Cecil /UK
16	Vortex	CABB/China

2.3 Methods

2.3.1 Determination of Urea

A. Principle:

Enzymatic and colorimetric method based on the specific action of urease which hydrolyses urea in ammonium ions and carbon dioxide Ammonium ions then form with chloride and salicylate a blue-green complex [93].

B. Assay Procedure:

Table (2-3) Assay procedure of urea

	Blank	Standard	Sample
Working Reagent (R1+R2)	1ml	1ml	1ml
Standard	_	5µl	-
Sample	-	-	5 µl
Demineralization water	5µl	-	-
Mix and wait for 4 minutes at room temperature or 2 minutes at 37°C			
Base (vial R3) diluted ¼	1ml	1ml	1ml
Content was mixed .Let stands for 8 minutes at room temperature or 5 minutes at 37°C. Read absorbance at 600 nm against blank.			

C. Calculations

Urea (mg/dl) =*A* Sample/ A Standard∗40

2.3.2 Determination of Creatinine

A. Principle:

Creatinine in a basic picrate solution forms a colored complex. The Δ extinction at predetermined times during conversion is proportional to the concentration of creatinine in the sample[94].

B. Assay Procedure

Table (2-4) Assay procedure of creatinine

	Blank	Standard	Sample
Stander	-	100µl	-
Sample	-	-	100µl
Working Reagent (R1+R2)	1ml	1ml	1ml

Content was mixed. After 30 seconds, absorbance A1 at 492 nm was recorded. After 1 min the first reading, was recorded absorbance A2.

C. Calculations

Creatinine (mg/dl) =((A2-A1)Assay/ (A2-A1) Standard)*2

2.3.3 Estimation of Glomerular Filtration Rates (GFR)

The eGFR is a useful and precise indicator of renal function and is calculated using serum creatinine as well as age, race and gender variables. The most frequently used assessment equation is according to Cockcroft – Gault equation [95]:

eGFR (mL/min/1.73 m^2) = (140-Age) *BMI (kg)*0.85 if female /72*Cr.(serum Mg/dl)

2.3.4 Determination of Albumin

A. Principle:

In the buffered solution at PH 4.2 bromocresol green binds albumin to form a colored compound which absorbance measured at 630 nm (620-640) is proportional to the albumin concentration in the specimen[96].

B. Assay Procedure

Table (2-5) Assay procedure of albumin

Reagent R1	1000µL
Blank, Standard ,Control or Specimen	5 µL
Content was mixed well and read absorbance at 630nm against blank after 3 minutes	

C. Calculation

Albumin (g/dL) = Abs(Assay) /Abs (Standard) *Standard concentration

2.3.5 Determination of Serum Glucose

A. Principle:

Trinder Method. Glucose is oxidized by Glucose oxidase (GOD) to gluconic acid and hydrogen peroxide which in conjunction with Peroxidase (POD), reacts with chloro phenol and PAP to form a red quinoneimine. The absorbance of the colored complexes, proportional to the concentration of glucose in the specimen[97] **.**

B. Assay Procedure:

Table (2-6) Assay procedure of glucose

	Blank	Standard	Sample
Stander	-	10µl	-
Sample	-	-	10µl
Working reagent	1ml	1ml	1ml

The content of tubes were mixed well and incubated for 10 minutes at 37°C. The absorbance (A) of standard and sample was read against the blank at 500 nm .

C. Calculations

Glucose (mg/dl) = A Sample /A Standard *100

2.3.6 Determination Total and Ionized Calcium Concentration

The serum calcium(total and ionized)were determined by using electrolyte analyzer instrumentation. This method is dependent upon the ion selective electrode (ISE) principle.

- **Principle**:

An ion-selective electrode (ISE) is an indicator electrode that responds (produces a potential) when it is placed in a solution containing a certain ion. There is now a large variety of ISE available which selectively respond to particular cations and anions, and certain gases. The ion-sensing part consists of semi permeable membrane (plastic) which has sites capable of adsorbing the analytic ion.

On other side of the membrane there is a solution containing the ion of interest: one of these is the test solution, the other is a standard solution within the electrode itself. Inside the electrode body there is an electrical connection electrode – to monitor the response from the membrane. The relationship of the tested ion concentration and electrodes electric potential was described in following equation [64].

E=Eo+ (RT/F)*Ln (ax)… where:

E: Electric potential of ion selective electrode during test

Eo: Standard electric potential of ion selective electrode

R: Gas constant (8.314 j/.mol)

T: absolute temperature (t+273 ºC)

F: Faraday constant (96487 C/mol)

Ax: Tested ions concentration in solution

The components of electrolytes kits and standard as show in Table (2-7):

Table (2-7): Components of electrolytes kit

	Component	Volume
Cleaning solution	Nonionic Surface active	250 ml
Ca^{++}(m mol/L)	2.5	1.5
DE protein solution	Diluent:0.1mol/L HCL	18 ml
	Protease: powder	100 mg
Electrolyte reagent DS-l	Standard A	390 ml
	Standard B	90 ml

- **Procedure:**

1. The instrument was turned on
2. Self-calibration was done by instrument.
3. From main screen, measure sample icon was selected.
4. Serum option was selected.
5. Serum sample was put under aspirated needle and sampling icon was pressed.

6. A volume of 160 µl was aspirated by needle; the result appeared on the screen and automatically for total Ca, Ca^{++} after 50 second.

7. Deprotinization and cleaning of instrument were undertaken after approximately 30 samples.

8. Internal quality control was carried out before starting analysis of samples and within batch of analysis to check the accuracy of the results.

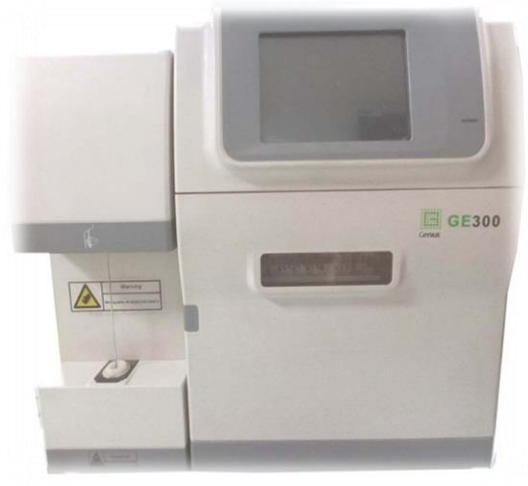


Figure (2-2): Electrolyte Analyzer (Genrui E300)apparatuses to measure calcium ions

2.3.7 Determination of Beta Trace Binding Protein by Enzyme Linked Immunosorbent Assay (ELISA)

- **Principle:**

The quantitative ELISA (Sandwich technique) was used in present study the kit's micro titer strip wells have been coated with an A monoclonal antibody specific for human BTP. The samples and standards are placed in the wells labeling and human BTP binds to the immobilized antibody. After eliminating unbound compounds with a wash phase, an

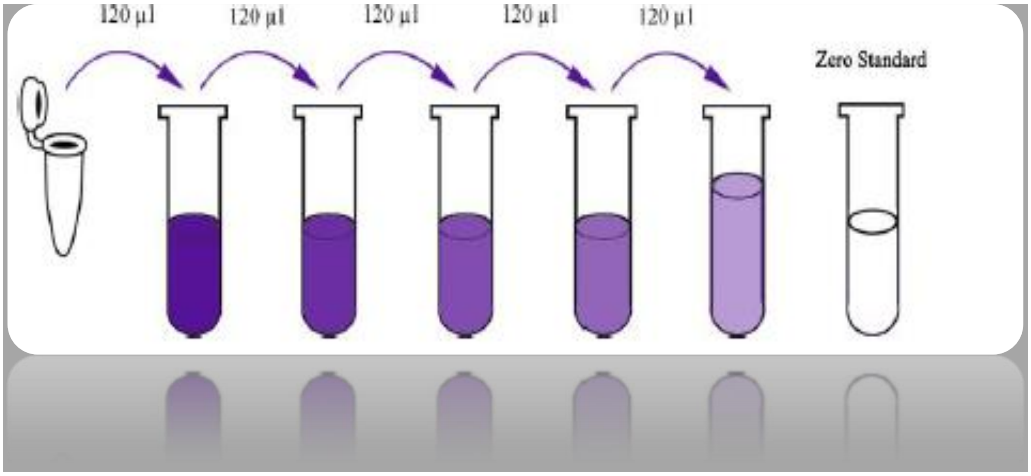
HRP-conjugated anti-human BTP antibody is added, which binds to human BTP caught by the Ab. During a wash step after incubation, unbound HRP-conjugated anti-human BTP antibody is removed. The wells are next filled with a chromogenic substrate solution, and color develops in proportion to the amount of BTP bound in the first phase. The reaction is completed when a colorful product is generated and a stop solution is added. At (450 nm), the color strength is determined spectrophotometrically [11].

- **Standard Preparation:**

A volume of 120µl standard (4800 µg/dl) with 120µl of standard diluent was mixed to generate a 2400 µg/dl standard stock solution. Then the standard leaved to sit for 15 mins with gentle agitation prior to making dilutions, serially diluting the standard stock solution (2400µg/dl) 1:2 with standard diluent to produce 1200µg/dl, 600 µg/dl, 300 and 150µg/dl solutions. Standard diluent serves as the zero standards (0 ng/L). Dilutions of standard solutions suggested were as follow in Table (2-8) and figure (2-3).

Table (2-8): Standard preparation steps for Beta trace protein

2400µg/dl	Standard No.5	120µl Original Standard + 120µl Standard diluent
1200	Standard No.4	120µl Standard No.5 + 120µl Standard diluent
600 µg/dl	Standard No.3	120µl Standard No.4 + 120µl Standard diluent
300 µg/dl	Standard No.2	120µl Standard No.3 + 120µl Standard diluent
150 µg/dl	Standard No.1	120µl Standard No.2 + 120µl Standard diluent



Standard Concentration	Standard No.5	Standard No.4	Standard No.3	Standard No.2	Standard No.1
4800 µg/dl	2400 µg/dl	1200 µg/dl	600 µg/dl	300 µg/dl	150 µg/dl

Figure (2-3): Concentration of standard Beta trace protein

- **Procedure E4141Hu:**

1. Firstly all reagents, standard solutions and samples were prepared. The assay is performed at room temperature.
2. A volume 50µl of standard solution was added to standard well.
3. 40µl samples was added to sample wells then 10µl anti-BTP antibody was added to sample wells after that 50µl streptavidin-HRP was added to sample wells and standard wells (Not blank control well). After that plate was mixed then incubated at 37°C for 60 minutes.
4. The sealer was removed and the plate was washed 5 times with wash buffer. Soak wells with at least 0.35ml wash buffer for 30 sec to 1 minute for each wash. For automated washing, were aspirated all wells and washed 5 times with wash buffer, overfilled wells with wash buffer. Blotted the plate onto paper towels or other absorbent materials.

5. A volume of 50µl substrate solution was added to each well. Plate was incubated covered with a new sealer for 10 minutes at 37°C in the dark place.
6. To each well a volume of 50µl stop solution was added, the blue color had been will change into yellow immediately.
7. The optical density (OD value) of each well was determined immediately by using a micro plate reader set to 450 nm within 10 minutes after added the stop solution.

Figure (2-4): Elisa instrumentation for BTP measurement

- **Calculation:**

A standard curve was plotted for the absorbance of each standard on the y-axis versus the concentration of these standards on the x-axis .In order to estimate the BTP concentration of each sample, first the horizontal line to the standard curve was extended, the point of intersection was found, a vertical line to the x-axis was extended and the corresponding sample concentration was read as shown in figure (2-5).

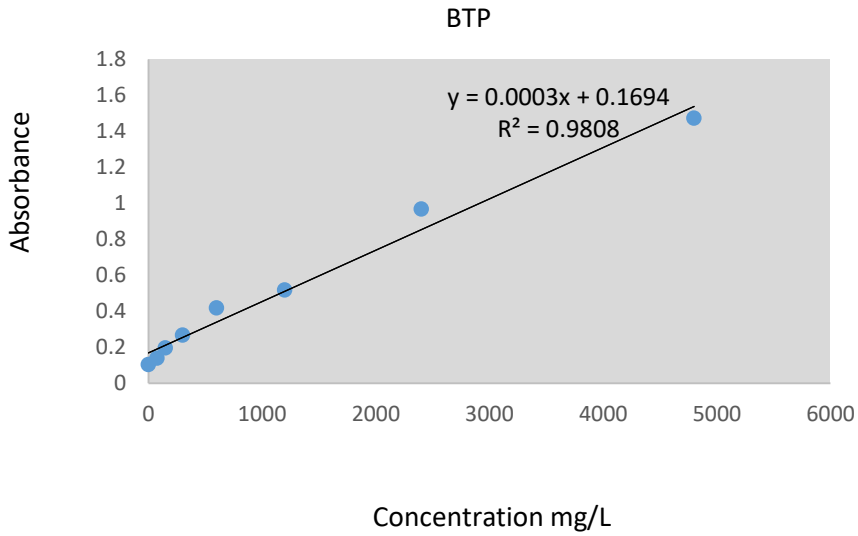


Figure (2-5) Standard curve for calculation of beta trace binding protein

2.3.8 Determination of Liver Fatty Acid Binding Protein by Enzyme Linked Immunosorbent Assay(ELISA)

- **Principle:**

The quantitative ELISA (Sandwich technique) was used in present study the kit's micro titer strip wells have been coated with an A monoclonal antibody specific for human L-FABP. The samples and standards are placed in the wells labeling and human L-FABP binds to the immobilized antibody. After eliminating unbound compounds with a wash phase, an HRP-conjugated anti-human L-FABP antibody is added, which binds to human L-FABP caught by the Ab. During a wash step after incubation, unbound HRP-conjugated anti-human L-FABP antibody is removed. The wells are next filled with a chromogenic substrate solution, and color develops in proportion to the amount of L-FABP bound in the first phase. The reaction is completed when a colorful product is generated

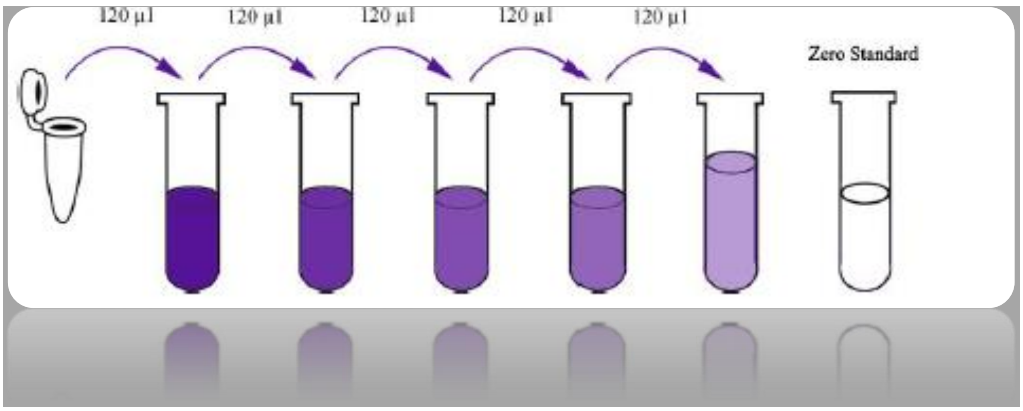
and a stop solution is added. At (450 nm), the color strength is determined spectrophotometrically[12].

- **Standard Preparation:**

A volume of 120µl standard (1920 ng/L) with 120µl of standard diluent was mixed to generate a 960 ng/L standard stock solution. Then the standard leaved to sit for 15 mins with gentle agitation prior to making dilutions, serially diluting the standard stock solution (960ng/L) 1:2 with standard diluent to produce 480 ng/L, 240ng/L, 120ng/L and 60ng/L solutions. Standard diluent serves as the zero standards (0 ng/L). Dilutions of standard solutions suggested were as follow in Table (2-9) and figure (2-6):

Table (2-9): Standard preparation steps for liver fatty acid binding protein

960µg/dl	Standard No.5	120µl Original Standard + 120µl Standard diluent
480 ng/L	Standard No.4	120µl Standard No.5 + 120µl Standard diluent
240 ng/L	Standard No.3	120µl Standard No.4 + 120µl Standard diluent
120ng/L	Standard No.2	120µl Standard No.3 + 120µl Standard diluent
60 ng/L	Standard No.1	120µl Standard No.2 + 120µl Standard diluent



Standard Concentration	Standard No.5	Standard No.4	Standard No.3	Standard No.2	Standard No.1
1920ng/L	960 ng/L	480ng/L	240ng/L	120 ng/L	60ng/L

Figure (2-6): Concentration of standard liver fatty acid binding protein

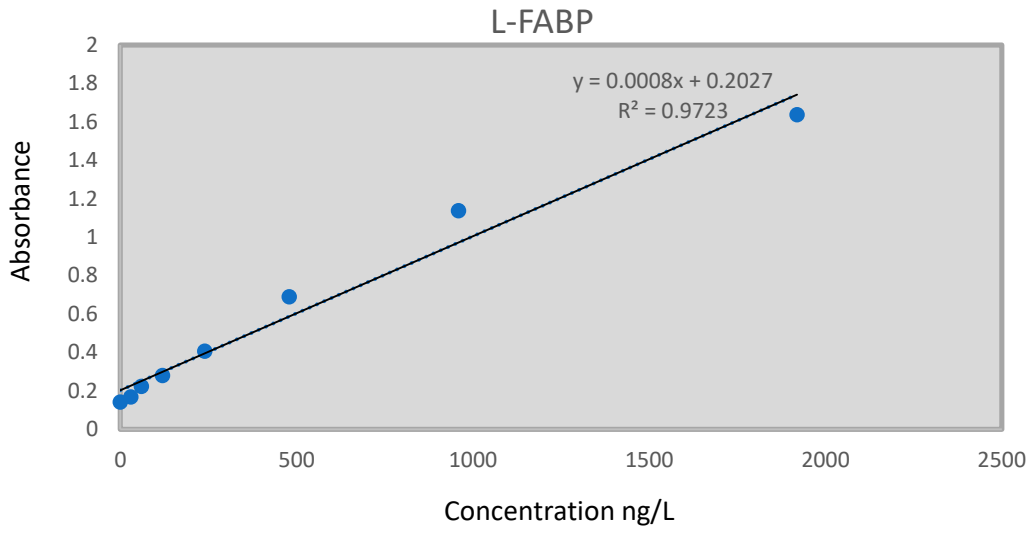
- **Procedure E 2159 Hu:**

1. Firstly all reagents, standard solutions and samples were prepared. The assay is performed at room temperature.
2. A volume 50µl of standard solution was added to standard well.
3. 40µl samples was added to sample wells then10µl anti-L-FABP antibody was added to sample wells after that 50µl streptavidin-HRP was added to sample wells and standard wells (Not blank control well).After that plate was mixed and covered with a sealer then incubated at 37°C for 60 minutes.
4. The sealer was removed and the plate was washed 5 times with wash buffer. Soak wells with at least 0.35ml wash buffer for 30 sec to 1 minute for each wash. For automated washing, aspirate all wells were aspirated and washed 5 times with wash buffer, overfilled wells with wash buffer. Blotted the plate onto paper towels or other absorbent materials.

5. A volume of 50µl substrate solution was added to each well. Plate was incubated covered with a new sealer for 10 minutes at 37°C in the dark place.
6. To each well a volume of 50µl stop solution was added, the blue color had been will change into yellow immediately.
7. The optical density (OD value) of each well was determined immediately by using a micro plate reader set to 450 nm within 10 minutes after adding the stop solution.

- **Calculation :**

A standard curve was plotted for the absorbance of each standard on the y-axis versus the concentration of these standards on the x-axis .In order to estimate the L-FABP concentration of each sample , first the horizontal line to the standard curve was extended, the point of intersection was found, a vertical line to the x-axis was extended and the corresponding sample concentration was read as shown in figure (2-7).



Figure(2-7)Standard curve for calculation of liver fatty acid binding protein

2.3.9 Estimation of Hydroxyl of Carboxyl and Amide Bands by Attenuated Total Reflection-Fourier Transform Infrared (ATR-FTIR) Spectroscopy

Principle:

Spectra were recorded in the 4000–600 cm^{-1} region using FTIR spectrometer (Bruker) Tensor27as in figure (2-8), fitted with an ATR unit with a diamond disc as an internal-reflection element. In the diamond the infrared beam is reflected at the interface towards the sample. The volume of saliva sample was 2 µL. The sample was dried on ATR crystal for 3 min and then the spectra of saliva film were recorded. The spectrum of air was used as a background. Background and sample spectra were taken with 4 cm^{-1} of resolution and 32 scans [98][99].

- **Procedure:**

For IR measurement saliva, A healthy, early and end stages kidney disease patients and serum samples were collected from the participants. about 2 µl of the sample transferred to the ATR unite and let it dry for 5 minutes then measure IR using Bruker Tensor 27- FTIR spectroscopy with ATR attachment and operated by OPUS 7.2 software (Bruker Optik GmbH) Table (2.2) **.** A background absorption spectrum for atmospheric and environment correction was taken before each measuring of the sample. Applied the sample on the crystal of ATR then start measuring the sample. The crystal washed every time with 70% absolute ethanol after each sample and drying with tissue paper. While after serum sample we used absolute ethanol for washing. The spectra were obtained in the range of 400 to 4000cm^{-1}, with a resolution of 4 cm^{-1}, with 15 scans and a measurement time of 15 seconds per spectrum. The ambient temperature

was 25°C. The spectrum was collected by the software opus program for analysis of the Bands.

Figure (2-8) Carton picture of FTIR spectrometer

2.4 Statistical Analysis:

Statistical analysis was done by using Software Package for Social Science SPSS version 26. The normality of data distribution was tested by the Kolmogorov-Smirnov and Shapiro-Wilk tests. Present data were Non-normally distributed. Mann-Whitney U test was applied for comparison between two groups that expressed as a mean rank, while test statistic(mean rank difference) and Std. test statistic of Krusskal Wallis Post Hock test were used for multi comparison among studied group. Also spearman correlation was used to determine the presence of correlation between data variance. The ROC analysis was used to determine the diagnostic markers for studied variables. The sensitivity and specificity of biochemical parameter calculate the optimal cutoff according to "Youden Index" by select the point that is closest to the top-left corner of the ROC curve giving equal weight to sensitivity and specificity when picking a cut-off point is a typical practice. This idea is often referred to as the Youden Index. The area under the curve (AUC) provides a useful tool to compare different biomarkers as Table (2-10). $P \leq 0.05$ consider as significant value[100].

Table (2-10) List of AUC ranges and their classification levels [100]

AUC Range	Classification Level
0.90 - 1.00	Excellent
0.80 - 0.90	Good
0.70 - 0.80	Fair
0.60 - 0.70	Poor
0.50 - 0.60	Failure

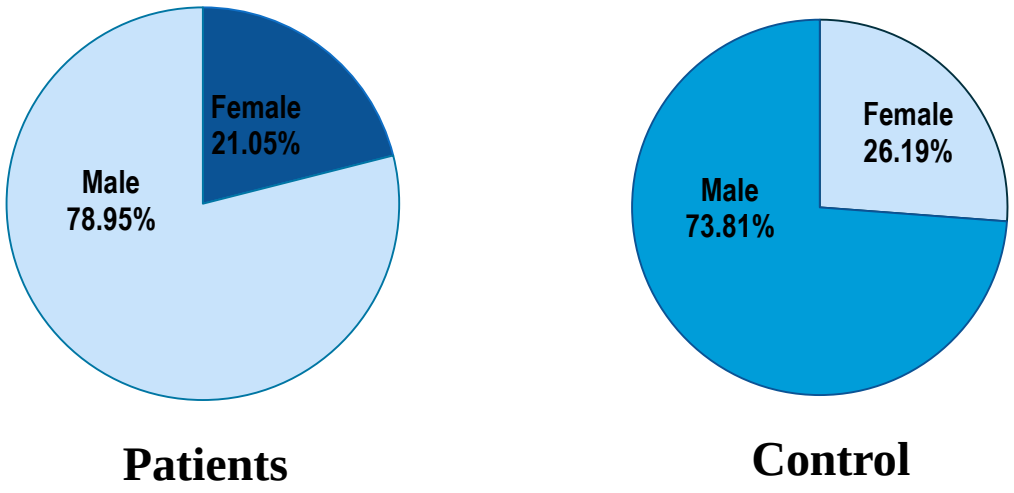
3.1Demographic Characterization of Studied Individuals

Table (3-1) have been shown non-significant changes (P >0.05) of study variables including age and BMI in studied groups (patients with kidney disease and control, median for age and BMI for patients were (55,24) with minimum (25,19) and maximum (63 ,29) levels respectively and the median of age and BMI for control group (52, 24) with minimum (25,18) and maximum (52, 29) levels respectively. While gender was described in figure (3-1) among studied groups, in patients there were(21.05%)female and (78.95%)male, while in control were (26.19%) female and (73.81%) in male.

Table (3-1) Demographic data of study and control groups using Mann-Whitney U test

	Patients N=(38)			Control N=(42)			
Variable	**Median**	**Minimum**	**Maximum**	**Median**	**Minimum**	**Maximum**	**P value**
Age (years)	**55**	**25**	**63**	**52**	**25**	**52**	**0.713**
BMI (kg/m^2)	**24**	**19**	**29**	**24**	**18**	**29**	**0.534**

N(number),Sig(significant), the significant level is ≤0.05 ,BMI (Body mass index)



Figure(3-1) Gender distribution in studied groups

The age and BMI matching required to removing potential discrepancies in parameter findings that may occur due to the effects of these factors[101] [102]. In present study the male more than female because men are more prone to prostate diseases and stones. Regularly, epidemiological studies of disease prevalence show that obstructive renal failure may occur more often in men[103].

3.2 Biochemical Variables Results

3.2.1 Estimation of Renal Function Tests

The biochemical changes observed in the studied groups were presented in (Table 3-2). The results indicated a significant (P <0.05) increase in the levels of urea and creatinine in both CKD and AKI groups compared to the control group. The mean rank differences were 44.474 and 35.526 respectively for urea, and 47.658 and 32.342 respectively for creatinine.

On the other hand, the albumin levels were significant (P <0.05) decreased in both CKD and AKI groups compared to the control group, the mean rank difference were (-18.596, -35.991)respectively. Additionally, the glomerular filtration rate (GFR) was significant(P < 0.05) decreased in the CKD group compared to both the AKI and control groups. The mean rank difference were (-18.789, -49.395) respectively. Furthermore GFR showed a highly significant difference when comparing the AKI and control groups with a mean difference of (-30.603).

Table (3-2) Krusskal Wallis Post Hock multi-Comparison of renal function tests of studied groups

Groups	CKD-AKI (N=38)			CKD-Control (N=61)			AKI-Control (N=61)		
	Mea rank difference	Std. Test Statistic	P value	Mea rank difference	Std. Test Statistic	P value	Mea rank difference	Std. Test Statistic	P value
Urea (mg/dl)	8.947	1.188	.235	44.474	6.932	.000	35.526	5.537	.000
Creatinine (mg/dl)	15.316	2.033	.042	47.658	7.422	.000	32.342	5.037	.000
GFR (ml/min/1.73m)	-18.789	-2.494	.003	-49.395	-7.693	.000	-30.605	-4.766	.000
Albumin (g/dl)	17.395	2.3	.06	-18.59	-2.89	.01	-35.99	-5.60	.000

CKD(chronic kidney disease), AKI(acute kidney disease) ,GFR(glomerular filtration rate)

The results for the levels of total calcium, ionized calcium and glucose in the studied groups are presented in (Table 3-3). The findings showed a significant decrease (P< 0.05) in both CKD and AKI groups when compared to the control group. The mean rank differences were -30.991 and -16.728 respectively for total calcium, (-18.368, -35.901and -17.533) respectively for ionized calcium. Additionally, the glucose levels were significantly increased (P <0.05) in both CKD and AKI groups, compared to the control group. The mean rank differences were (29.782 and 25.256) respectively.

Table (3-3) Krusskal Wallis Post Hock multi-Comparison of (total and ionized) calcium and glucose of studied groups

Groups	CKD-AKI (N=38)			CKD-Control (N=61)			AKI-Control (N=61)		
	Mea rank difference	Std. Test Statistic	P value	Mea rank difference	Std. Test Statistic	P value	Mea rank difference	Std. Test Statistic	P value
Total calcium(mg/dl)	-14.263	-1.895	.058	-30.991	-4.831	.000	-16.728	-2.608	.009
Ionized calcium(mg/dl)	-18.368	-2.441	.015	-35.901	-5.598	.000	-17.533	-2.734	.006
Glucose(mg/dl)	4.526	.601	1.00	29.782	4.641	.000	25.256	3.935	.000

CKD(chronic kidney disease), AKI(acute kidney disease)

As in Table (3-3) results of present study were agreement with previous study that recorded the urea and creatinine were increase and the GFR decrease as a result of the kidney ability to function declines gradually and over time in chronic renal failure, the reason may be attributable to

hypertension, diabetes or some other age relate[104][105] . In spite of serum urea is not recommended for routine assessment of renal function because it is a less specific marker of glomerular filtration rate (GFR) than serum creatinine .And it is a side effect of a more serious medical problem but it is still use as a part of renal function test . Actually, the kidney disease improving Global Outcomes (KDIGO) statement states that CKD is indicated by a GFR of less than 60 mL/minute/1.73 m^2. One of the main factors contributing to renal failure is high blood pressure, it is regulated by kinases that are Ca^{2+} dependent and G-protein coupled [106], the kidney's blood arteries might be harmed by hypertension, which would also affect how much waste is secreted. Hypocalcemia (total and ionized) that recorded in present study consider as one of the late event in CKD[107]. Also DM is the major cause of renal morbidity and mortality, and diabetic nephropathy is one of chronic kidney failure. Good control of blood glucose level is absolute requirement to prevent progressive renal impairment[108]. Results of glucose in present study indicate increase of glucose CKD group that indicated the effect of it in development of renal failure that may be referred to glucose transporters that play an important role in renal functioning (GLUT proteins (Glucose transport protein) and sodium-dependent glucose transporters (SGLT)). These membrane transport proteins release glucose derived from renal gluconeogenesis. These disturbance may illustrated development of diabetic nephropathy [109].

3.2.2 Level of Beta Trace Protein (BTP)and Liver Fatty Acid Binding Protein(LFABP) in Studied Groups

In Table (3-4) and Figure (3-2), it can be seen that BTP levels significantly increased (P < 0.05) in CKD patients, compared to both AKI and the control group. The mean rank differences were 21.974 and 20.814, respectively. Otherwise in Table (3-4) and Figure (3-3), it can also be observed that LFABP levels significantly decreased (P <0.05) in AKI patients, compared to both CKD and the control group. The mean rank differences were 21.222 and -15.306, respectively.

Table (3-4) Krusskal Wallis Post Hock multi-Comparison of BTP and L-FABP in studied groups

Groups	CKD-AKI (N=38)			CKD-Control (N=61)			AKI-Control (N=61)		
	Mea rank difference	Std. Test Statistic	P value	Mea rank difference	Std. Test Statistic	P value	Mea rank difference	Std. Test Statistic	P value
Beta trace protein (mg/dl)	21.974	2.989	0.003	20.814	3.297	0.001	-1.160	-0.184	0.854
Liver fatty acid binding protein(ng/L)	21.222	2.846	0.004	5.916	0.935	0.350	-15.306	-2.420	0.016

CKD(chronic kidney disease), AKI(acute kidney disease)

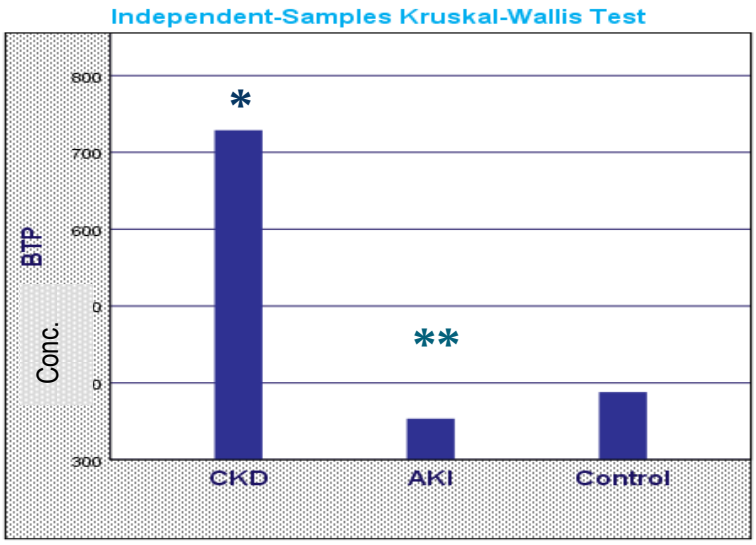


Figure (3-2) mean rank for BTP in studied group

***Significant difference between CKD and AKI**
****Significant difference between CKD and control**

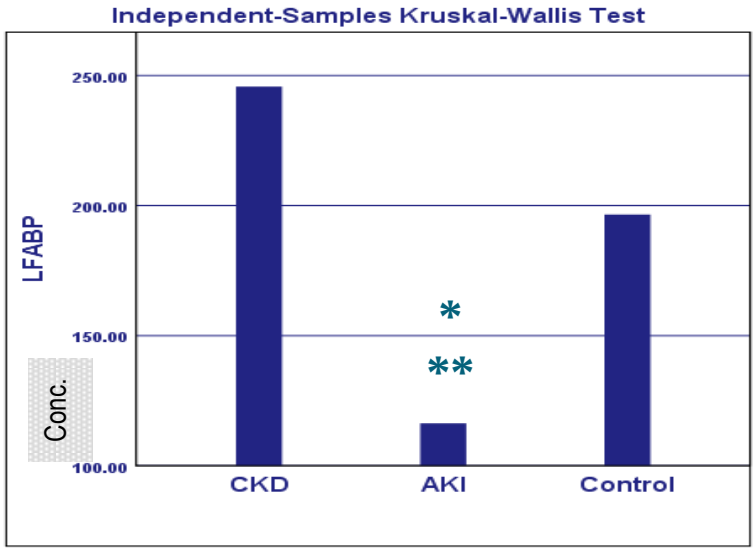


Figure (3-3) mean rank for LFABP in studied groups

*** Significant difference between AKI and CKD**
**** Significant difference between AKI and Control**

Recently, BTP can be used as a biomarker of kidney damage alternative to albuminuria as it may detect renal injury earlier than albuminuria because of its lower molecular mass, constant production rate, ionic property, and its stability[72][110]. In current study, attempt to estimate it to determine its role in detection of renal damage. It has been shown that BTP increase in CKD group mainly. This result was expected because BTP is a low molecular weight glycoprotein consider as an emerging novel marker of glomerular filtration rate. Also, BTP can detect mild deterioration in renal function , unlike serum creatinine , also the BTP levels are less affected than creatinine by age and sex and ethnicity. In addition that cystatin C andβ2 microglobulin (B2M) levels may be affected by inflammation. All these findings are important for the use of GFR estimating equations based on low-molecular-weight serum proteins throughout the range in GFR[111][112]. Actually one of the most important finding was recorded that BTP seem less affected by non-renal determinants. Mainly this evidence is mostly based on the studies including patients with chronic kidney disease. Herein all of these observations were synergistically with present finding about BTP that consider as a good marker for developing renal impairment from early stage to end stage[70]. Another recent markers was examined in present work is L-FABP, it is expressed in the proximal tubules of the human kidney and participates in fatty acid metabolism[113]. In past clinical study, urinary excretion of L-FABP was reported to offer potential clinical marker to screen for kidney dysfunction and thereby to identify patients who are likely to experience deterioration of renal function in the future L-FABP transports free fatty acids to organelles such as the mitochondria and lysosomes for β-oxidation for use in these cellular processes[114]. Actually, tubular interstitial damage

was decreased by the expression of human L-FABP in renal proximal tubules. Therefore, urinary L-FABP will be a novel biomarker to detect the progression of renal interstitial injury[115]. In present study L-FABP was decreased significantly in acute kidney injury when compared with chronic and control groups this result illustrated the ability of kidney to excrete LFABP that indicated earlier renal alteration function in acute kidney stage, furthermore LFABP increase in CKD patients of present study that main referred to inability of kidney in this stage to excrete urine and developing of oliguria [116]. Also the underlying causes of over expression of L-FABP are hypoxia in tubular cells and anemia which increase urinary excretion of L-FABP [117] [118].

Therefore the LFABP as clinical indicator tissue injury marker, after cell damage small proteins diffuse more rapidly than large proteins through the interstitial space via endothelial clefts into vascular space. The size of these endothelial clefts is variable, from large clefts in the liver to smaller pores in the heart, the skeletal muscle and finally to almost complete impermeability in the brain (blood–brain barrier). As a result, the diffusion rate of the released proteins into the circulation also differs. Therefore, the time of appearance of these marker proteins in plasma is not only dependent on the time course of the disease, but also on molecular size and distribution over extravascular compartments [119]. These finding with present results may need further studies to examine the relationships between levels in serum and urine to determine the exact effect.

3.2.3 Estimation of Salivary Hydroxyl and Amide Bands in Studied Groups by Using Attenuated Total Reflection-Fourier Transform Infrared (ATR-FTIR) Spectroscopy

Based on the data obtained from FT-IR (Bruker) Tensor27 analysis, the following observations can be made in CKD patients, the salivary amide and hydroxyl bands show a significant decrease ($p<0.05$) when compared to both AKI patients and the control group. The average mean rank differences for amide bands were -17.421 and -15.227 respectively, as presented in Table (3-5) and figure (3-4). For hydroxyl bands, the average mean rank differences were -22.711 and -34.689 respectively, as shown in Table (3-5) and figure (3-5).Also, the decrease in hydroxyl and amide bands shown in figures (3-6) (3-7) (3-8).

Table (3-5) Krusskal Wallis Post Hock multi-Comparison of amide and hydroxyl in studied groups

Groups	CKD-AKI (N=38)			CKD-Control (N=61)			AKI-Control (N=61)		
	Mea rank difference	Std. Test Statistic	P value	Mea rank difference	Std. Test Statistic	P value	Mea rank difference	Std. Test Statistic	P value
Amide (cm^{-1})	-17.421	-2.311	.021	-15.227	-2.370	.018	-2.194	-.342	.733
Hydroxyl (cm^{-1})	-22.711	-3.012	.003	-34.689	-5.399	.000	-11.978	-1.864	.062

CKD(chronic kidney disease), AKI(acute kidney disease)

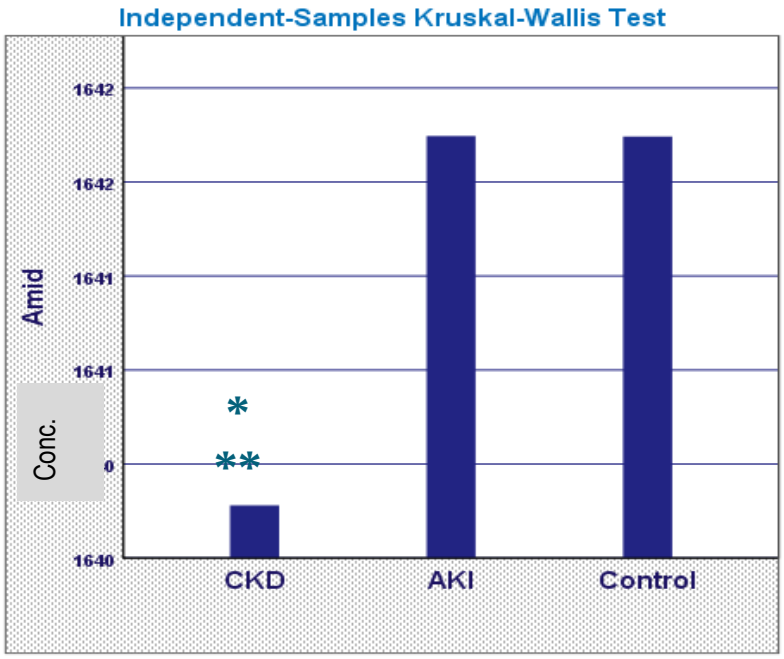


Figure (3-4) Salivary amide bands average rank of studied groups

***significant difference between CKD with AKI**

****Significant difference between CKD with control**

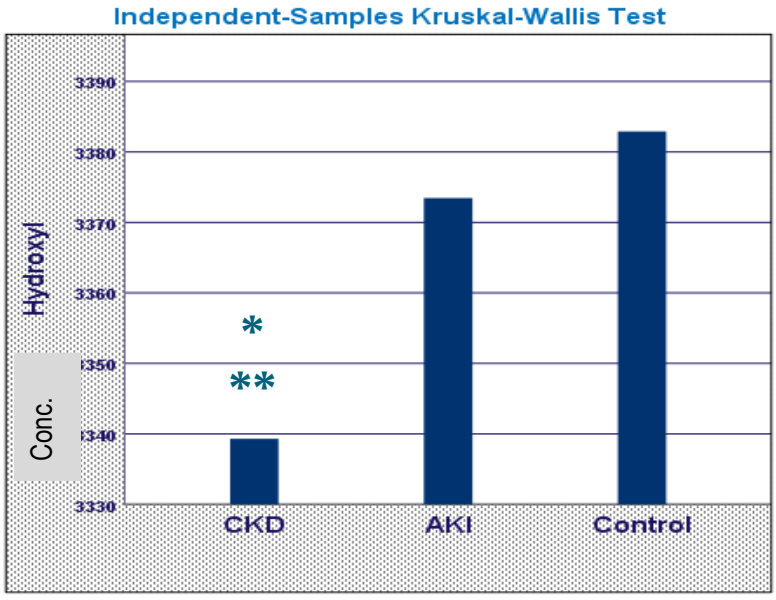


Figure (3-5) Salivary hydroxyl bands average rank of studied groups

***Significant difference between CKD and AKI**

****Significant difference between CKD and control**

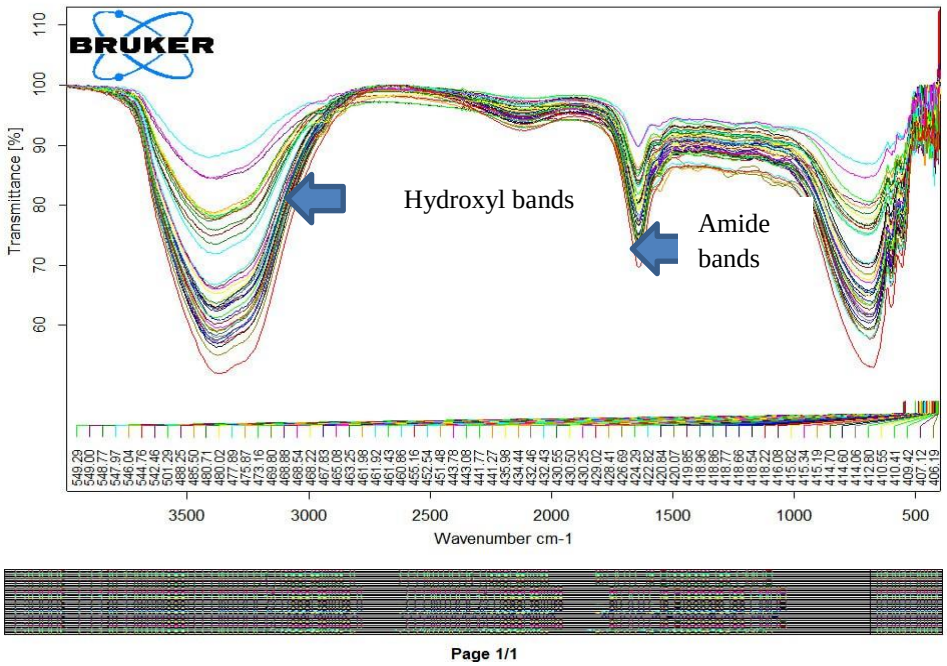


Figure (3-6)Carton picture of collection band of control

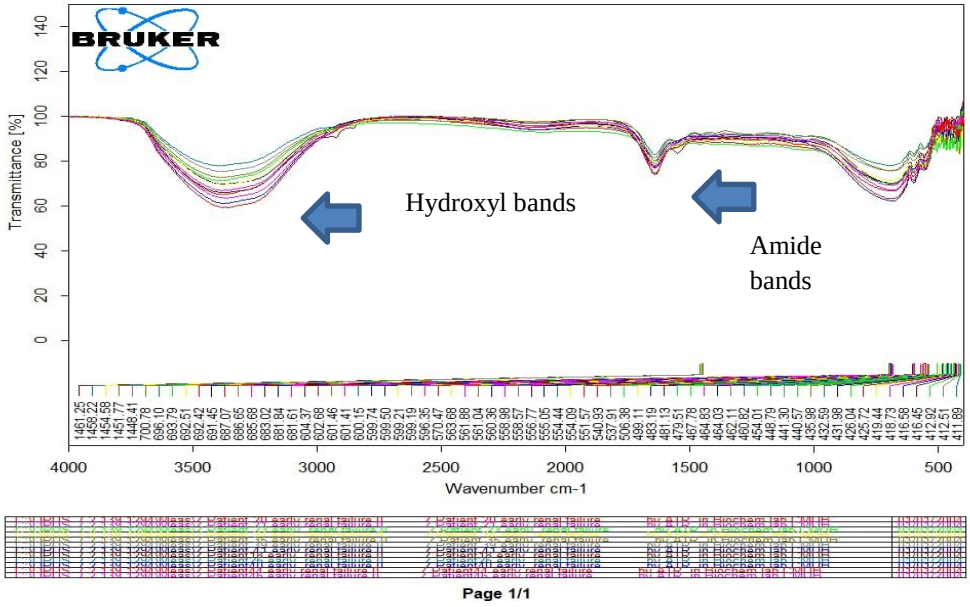


Figure (3-7) Carton picture of collection band of AKI

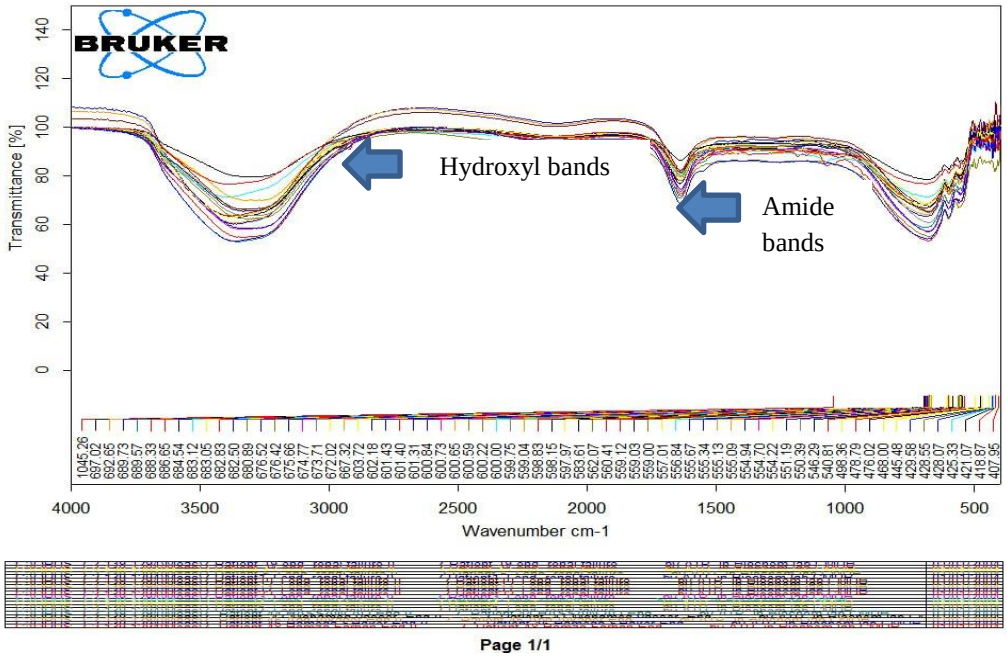


Figure (3-8) Carton picture of collection bands CKD

The use of saliva for the diagnosis of chronic kidney disease (CKD) through infrared spectroscopy is an emerging research area in clinical diagnostics. Infrared spectroscopy is a non-invasive technique that detects changes in the chemical composition of biological samples based on the unique spectral fingerprint of their molecular vibrations[122] .

Recent studies have suggested that saliva can provide valuable diagnostic information about kidney disease, as it contains various biomarkers that reflect renal function such as urea, creatinine, and albumin. Researchers have used infrared spectroscopy to analyze the salivary biomarker profiles of CKD patients and healthy controls and found that significant differences exist between the two groups [51].

In particular, the infrared spectra of saliva from CKD patients show characteristic changes in the intensity and shape of various spectral peaks,

indicating alterations in the levels of specific biomolecules. These changes are thought to be associated with the pathological changes that occur in the kidneys during CKD [123] .

In general CKD patients have dysfunction of salivary glands, which intensifies with the oxidation of salivary proteins/lipids. In CKD, pathological changes of oral mucosa, susceptibility to fungal infections and olfactory and taste disorders were observed[124] .Therefore, it is not surprising that saliva secretion was lower in all adult with CKD in comparison to AKI and controls. However, hypo salivation was observed only in patients with severe renal (4–5 stage CKD) that consider one of the difficulties during sample collection in present work [125]. From present data this infrared FTIR spectroscopy-based method opens a new level of non-invasive out of the laboratory diagnostics. The method makes it possible to determine the concentrations of the most important saliva substances without special sample preparation and reagents, from the minimum sample volume and (almost) immediately after the sample collection. Overall, the use of saliva for the diagnosis of CKD using infrared spectroscopy with ATR unite is a promising area of research that could offer a non-invasive and cost-effective alternative to traditional blood and urine tests Also, this aspects open new line for examination of salivary total protein, cortisol, a-amylase, sIgA, urea and phosphate concentration changes allow us to characterize the organism state regardless to age, physical fitness and stress level[126]. However, further studies are needed to validate the diagnostic accuracy and clinical utility of this approach.

3.3 Correlation Between Variables in Patients with Kidney Disease

Table (3-6) demonstrated results of the Spearman's correlation analysis, which indicates a significant ($p<0.05$) positive correlation between BTP and LFABP. Moreover, hydroxyl is a significant ($P <0.05$) positive correlation with amide and GFR. Conversely, a significant ($p<0.05$) negative correlation was observed between BTP and GFR as well as amide,and between LFABP with amide ,hydroxyl and GFR and between hydroxyl with LFABP and creatinine, also between amide with LFABP and BTP.

Table (3-6) The spearman correlation between variable among the patients group

Spearman's Correlation N(38)		LFABP	BTP	Hydroxyl	Amide	Glucose	urea	Creatinine	albumin	T.Ca	I.Ca	GFR
LFABP ng/l	Correlation Coefficient	1.000	.815	-.384	-.517	-.052	-.056	.202	-.020	-.069	-.122	-.350
	Sig.	.	.000	.021	.001	.762	.747	.239	.909	.688	.478	.036
BTP mg/dl	Correlation Coefficient	.815	1.000	-.288	-.372	.077	.045	.241	.253	-.092	-.136	-.414
	Sig.	.000	.	.080	.022	.645	.788	.146	.125	.584	.414	.010
Hydroxyl cm^{-1}	Correlation Coefficient	-.384	-.288	1.000	.564	-.082	-.220	-.327	-.028	.227	.309	.429
	Sig.	.021	.080	.	.000	.625	.185	.045	.866	.170	.059	.007
Amide cm^{-1}	Correlation Coefficient	-.517	-.372	.564	1.000	.233	.142	-.189	-.174	.243	.311	.211
	Sig.	.001	.022	.000	.	.159	.395	.257	.296	.141	.058	.203
Glucose mg/dl	Correlation Coefficient	-.052	.077	-.082	.233	1.000	.230	-.208	-.178	-.041	-.042	.140
	Sig.	.762	.645	.625	.159	.	.166	.210	.284	.805	.803	.400
Urea mg/dl	Correlation Coefficient	-.056	.045	-.220	.142	.230	1.000	.605	.102	-.126	-.093	-.581
	Sig.	.747	.788	.185	.395	.166	.	.000	.541	.453	.580	.000
Creatinine mg/dl	Correlation Coefficient	.202	.241	-.327	-.189	-.208	.605	1.000	.308	-.241	-.246	-.820
	Sig.	.239	.146	.045	.257	.210	.000	.	.060	.144	.136	.000
Albumin g/dl	Correlation Coefficient	-.020	.253	-.028	-.174	-.178	.102	.308	1.000	-.130	-.206	-.392
	Sig.	.909	.125	.866	.296	.284	.541	.060	.	.438	.216	.015
T.Ca mg/dl	Correlation Coefficient	-.069	-.092	.227	.243	-.041	-.126	-.241	-.130	1.000	.963	.331
	Sig.	.688	.584	.170	.141	.805	.453	.144	.438	.	.000	.043
I.Ca mg/dl	Correlation Coefficient	-.122	-.136	.309	.311	-.042	-.093	-.246	-.206	.963	1.000	.381
	Sig.	.478	.414	.059	.058	.803	.580	.136	.216	.000	.	.018

3.3.1 Correlation Between Beta Trace Protein and Glomerular Filtration Rate

The current study revealed a significant negative correlation (P value 0.01, r=0.414) between BTP and GFR. As with other renal markers, serum levels of BTP were highly associated with renal impairment in CKD stages, with its concentrations increasing significantly from stage 1 to 5. Previous investigations have demonstrated that BTP displays inverse a greater increase proportional with any changes in GFR at various stages of the disease. Furthermore, it has been observed that BTP can detect diminished GFR earlier than creatinine and is independent of age and gender, as indicated by prior research [127] [128] [129].

3.3.2 Correlation of Beta Trace Protein with Liver Fatty Acid Binding Protein

The results of current study express a significant positive correlation between the BTP and LFABP in kidney disease patients (P value =0.000 r=0.815). This considered as a suspected result because the two parameters mainly effected by renal damage before. This supported with previous study that indicated the BTP was significantly correlated with all the measured urinary renal markers, of the various proteins freely filtered, but reabsorbed in the proximal tubule[130]. The increase the BTP in the serum values of these reflects impaired renal ability to remove waste products from the body in patients with CKD. BTP are removed from the circulation mainly by renal filtration. Accordingly, in current study a strong correlation with GFR, BTP concentrations therefore they increased BTP with decreasing GFR .This result is agreement with previous study [131].

3.3.3 Correlation Between the Salivary Hydroxyl and Amide Bands

The current study shows a significant positive correlation between amide and hydroxyl bands (P value=0.000, r= 0.564). This confirm present explanation about salivary content of kidney, that's the functional groups of protein (hydroxyl in carboxyl and amide terminal) decrease in patients with chronic kidney disease.

3.4 Receiver Operating Characteristic (ROC)

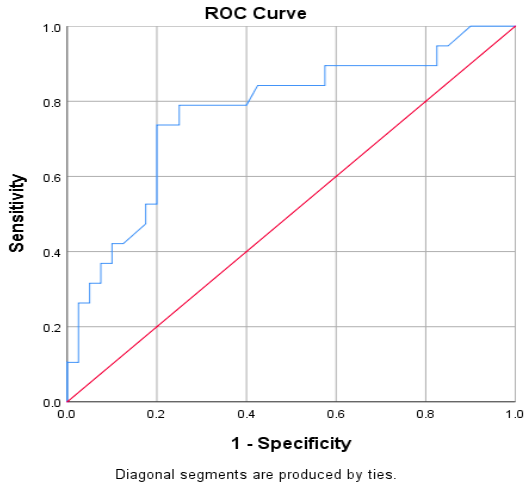
3.4.1 Receiver Operating Characteristic (ROC) Analysis of the Beta Trace Protein for Patients with Chronic Kidney Disease and Control

ROC curve for the sensitivity and specificity of BTP for CKD in comparison with control at (cut-off point was 363 µg/dl), AUC (Area under the curve) was 0.77and P value=0.001, the sensitivity and specificity were 84%, 58 %respectively, as shown in Table(3-7) Figure(3-9).

Table (3-7) Area under the curve of B TP between CKD and control

Area under the curve (AUC)	Sensitivity	Specificity	SE	P value	95% (Confidence interval)
0.77	**84%**	**58%**	**0.069**	**0.001**	**0.635-0.906**

SE (stander error)



Figure(3-9): Roc Curve of beta trace protein between CKD and control

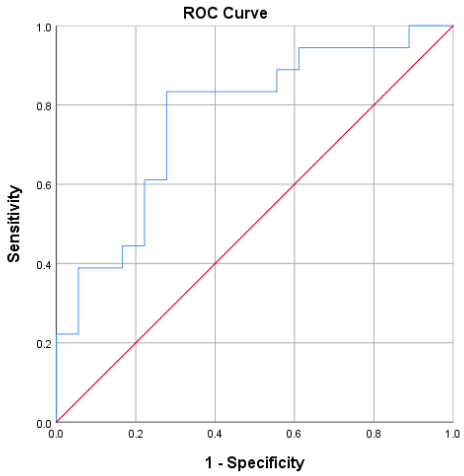
3.4.2 Receiver Operating Characteristic (ROC) Analysis of the Liver Fatty Acid Binding Protein for Chronic Kidney Disease and Acute Kidney Injury Patients

ROC curve for the sensitivity and specificity of liver fatty acid binding protein for CKD in comparison with AKI at (cut-off point was 121ng/L), AUC (Area under the curve)was0.76 the sensitivity and specificity were 83% ,73 % respectively, as shown in Table(3-8) and Figure(3-10).

Table (3-8) Area under the curve of LFABP between CKD and AKI

Area under the curve (AUC)	Sensitivity	Specificity	SE	P-value	95% (Confidence interval)
0.769	**83%**	**73%**	**0.080**	**0.006**	**0.611-0.926**

SE (standard error)



Figure(3-10): Roc Curve of Liver fatty acid binding protein between CKD and AKI

3.4.3 Receiver Operating Characteristic (ROC) Analysis of the Salivary Hydroxyl Band Between Patients with Chronic Kidney Disease and Control

ROC curve for the sensitivity and specificity of salivary hydroxyl for CKD in comparison with control at (cut-off point was3373.20 cm^{-1}), AUC (Area under the curve) was 0.911 and P value = 0.000, the sensitivity and specificity were 90%, 78 %respectively, as shown in Table (3-9) Figure (3-11) .

Table (3-9) Area under the curve of hydroxyl in carboxyl group band between control and CKD

Area under the curve (AUC)	Sensitivity	Specificity	SE	P-value	95%Confidence Interval
0.91	**90%**	**78%**	**0.046**	**0.000**	**0.820-1.000**

SE (standard error)

The discriminatory power for CKD of these salivary vibrational modes can reach AUC for hydroxyl 0.91 the sensitivity and specificity were 90% and 78% respectively, suggesting that salivary vibrational modes had a high capacity to discriminate patients with chronic kidney disease from healthy people.

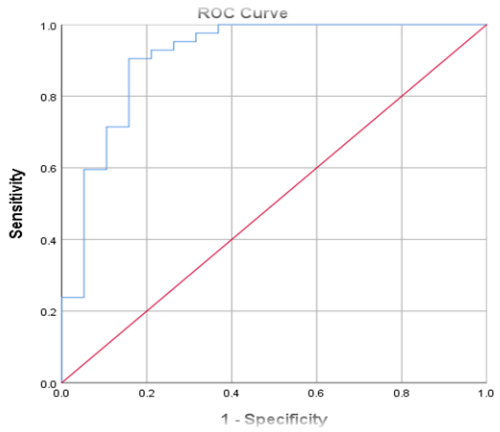


Figure (3-11) :Roc Curve of hydroxyl band between control and CKD

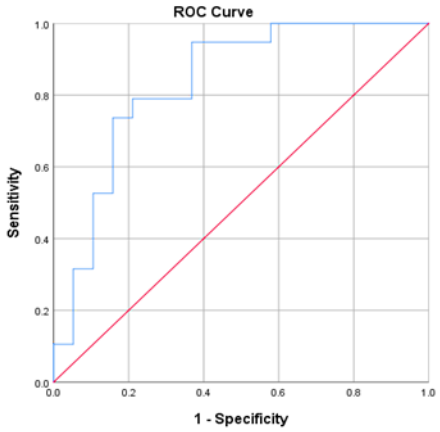
3.4.4 Receiver Operating Characteristic (ROC) Analysis of the Salivary Hydroxyl Band Between Patients with Chronic Kidney Disease and Patients with Acute Kidney Injury

ROC curve for the sensitivity and specificity of salivary hydroxyl for CKD in comparison with AKI at (cut-off point was3343cm^{-1}), AUC (Area under the curve) was 0.834 and P value = 0.000, the sensitivity and specificity were 94%, 64 %respectively, as shown in Table (3-10) Figure (3-12).

Table (3-10) Area under the curve of hydroxyl band between CKD and AKI

Area under the curve (AUC)	Sensitivity	Specificity	SE	P-value	95%Confidence Interval
0.834	**94%**	**64%**	**0.68**	**0.000**	**0.701-0.966**

SE (standard error)



Figure(3-12) :Roc Curve of hydroxyl band between CKD and AKI

Regarding with high sensitivity and level of (AUC) of salivary hydroxyl band OF CKD with both AKI and control may open new filed for use this marker in diagnosis of CKD that consider as speed, low cost and invasive test. This part need further studies with large sample size and standard conditions to use it in future**.**

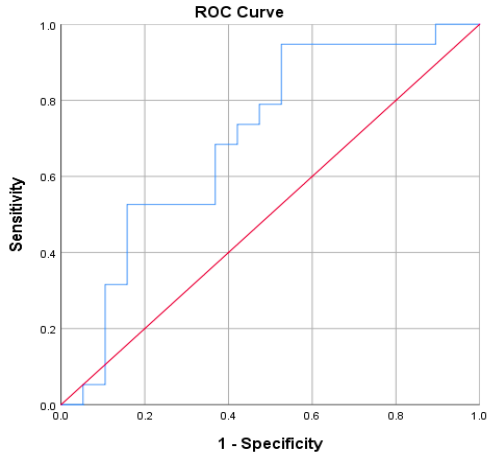
3.4.5 Receiver Operating Characteristic (ROC) Analysis of the Salivary Amide Band Between Patients with Chronic Kidney Disease and Acute Kidney Injury

ROC curve for the sensitivity and specificity of salivary hydroxyl for CKD in comparison with AKI at (cut-off point was1641cm^{-1}), AUC (Area under the curve) was 0.70 and P value = 0.034, the sensitivity and specificity were 68% ,64 %respectively, as shown in Table (3-11) Figure (3-13).

Table (3-11) Area under the curve of amide band between CKD and AKI

Area under the curve (AUC)	Sensitivity	Specificity	SE	P-value	95%Confidence Interval
0.701	**68%**	**64%**	**0.087**	**0.034**	**0.529-0.872**

SE (standard error)



Figure(3-13) :Roc Curve of amide band between CKD and AKI

Conclusions

1- The present results indicated that serum beta trace protein and liver fatty acid binding protein may consider a potential markers for detection and progression of kidney disease .

2- The changes of liver fatty acid binding protein in acute kidney injury group mainly make it useful in early detection of disease worsening.

3- The strong connection between beta trace protein and glomerular filtration rate (GFR) make it as a stabile protein for determination of GFR in kidney disease patients.

4- Present potential outcome of salivary hydroxyl vibrational mode was able to discriminate chronic kidney disease patients from healthy subjects.

5- Present data indicated that attenuated total reflection-fourier transform infrared (ATR-FTIR) spectroscopy based method open a new approach of noninvasive out of the laboratory diagnosis.

Recommendations

- Measurement of beta trace protein, liver fatty acid binding protein, urea ,creatinine and albumin in saliva as a complementary step for the detection of patients with renal disease.
- Measurement beta trace protein and liver fatty acid binding protein in urine.
- Large sample size are required to confirm the obtained results.

References

[1] W. L. and peter St., “Introduction Chronic Kidney Disease.” Supplement to Journal of Managed Care Pharmacy, p. 13;9, 2007.

[2] A. S. Levey and Eckardt, “Nomenclature for kidney function and disease: report of a Kidney Disease: Improving Global Outcomes (KDIGO) Consensus Conference,” *Kidney International* , vol. 97, no. 6, pp. 1117–1129, 2020.

[3] J.-C. Lv and L.-X. Zhang, “Prevalence and Disease Burden of Chronic Kidney Disease,” in *Renal fibrosis: mechanisms and therapies*, Springer, pp. 3–15, 2019.

[4] N.-E. Rasel, M. S. Alam, M. A. Zahan, and M. S. E. Hossain, “Serum creatinine can be reduced by applying homeopathic medicines according to the symptom similarity: Case study analysis of chronic kidney disease (CKD),” J. Complement. Altern. Med. Res., vol. 12, no. 1, pp. 1–10, 2020.

[5] T.-F. Chuang, H.-C. Hung, and SF Li, “Risk of chronic kidney disease in patients with kidney stones—a nationwide cohort study,” *BMC Nephrology* , vol. 21, pp. 1–7, 2020.

[6] A. Fadlelmoula, D. Pinho, V. H. Carvalho, S. O. Catarino, and G. Minas, “Fourier Transform Infrared (FTIR) Spectroscopy to Analyse Human Blood over the Last 20 Years: A Review towards Lab-on-a-Chip Devices,” *Micromachines*, vol. 13, no. 2, p. 187, 2022.

[7] E. den Bakker, R. J. B. J. Gemke, and A. Bökenkamp, “Endogenous markers for kidney function in children: a review,” *Crit. Rev. Clin. Lab. Sci.*, vol. 55, no. 3, pp. 163–183, 2018.

References

[8] M. Dajak, S. Ignjatovic, B. Stojimirovic, S. Gajic, and N. Majkic-Singh, "Evaluation of renal damage by urinary beta-trace protein in patients with chronic kidney disease," *Clin. Lab., vol. 57,* no. 1, p. 29, 2011.

[9] Y. Xu, Y. Xie, X. Shao, Z. Ni, and S. Mou, "L-FABP: A novel biomarker of kidney disease," *Clin. Chim. acta*, vol. 445, pp. 85–90, 2015.

[10] J. Rico-Fontalvo, G. Aroca-Martínez, and Daza-Arnedo, "Novel Biomarkers of Diabetic Kidney Disease," *Biomolecules*, vol. 13, no. 4, p. 633, 2023.

[11] N. Li, L. Han, X. Wang, O. Qiao, and Zhang, "Biotherapy of experimental acute kidney injury: emerging novel therapeutic strategies," *Transl. Res.*, 2023.

[12] M. Yuliari, H. Susianti, and D. A. Tandio, "Perbedaan Kadar serum Liver-Type Fatty Acid Binding Protein (L-FABP) dan Beta Trace Protein (BTP) Dalam Serum Sebelum dan Saat Cedera Ginjal Akut," *Intisari Sains Medis*, vol. 13, no. 2, pp. 377–380, 2022.

[13] T. Naber and S. Purohit, "Chronic kidney disease: Role of diet for a reduction in the severity of the disease," Nutrients, vol. 13, no. 9, p. 3277, 2021.

[14] R. Morcos , I Lazar, M Kucharik and A Lavin "The healthy, aging, and diseased kidney: relationship with cardiovascular disease," *J. Am. Geriatr. Soc.*, vol. 69, no. 2, pp. 539–546, 2021.

[15] T. Naber and S. Purohit, "Chronic Kidney Disease: Role of Diet for a

Reduction in the Severity of the Disease," *Nutrients*, vol. 13, no. 9, p. 3277, Sep. 2021.

[16] A. S. Levey, M. E. Grams, and L. A. Inker, "Uses of GFR and Albuminuria Level in Acute and Chronic Kidney Disease," *New. England. Journal. Medicine.*, vol. 386, no. 22, pp. 2120–2128, 2022.

[17] N. H. Lameire *et al.*, "Harmonizing acute and chronic kidney disease definition and classification: report of a Kidney Disease: Improving Global Outcomes (KDIGO) Consensus Conference," *Kidney International.*, vol. 100, no. 3, pp. 516–526, 2021.

[18] K. Makris and L. Spanou, "Acute kidney injury: definition, pathophysiology and clinical phenotypes," *Clin. Biochemist. Review.*, vol. 37, no. 2, p. 85, 2016.

[19] J. Gameiro, J. Agapito Fonseca, S. Jorge, and J. A. Lopes, "Acute kidney injury definition and diagnosis: a narrative review," *Journal of Clinical. Medicine.*, vol. 7, no. 10, p. 307, 2018.

[20] S. Nakanishi, M. Miyazato, R. Miyagi, and S. Saito, "Acute renal failure in a neonate due to bilateral primary obstructive megaureters," *J. Pediatr. Surg. case reports*, vol. 31, pp. 69–70, 2018.

[21] 1 and Chirag R. Parikh2 Jeremy Puthumana, 1 Heather Thiessen-Philbrook, 2 Leyuan Xu, 1 Steven G. Coca, 3 Amit X. Garg, 4 Jonathan Himmelfarb, 5 Pavan K. Bhatraju, 6 T. Alp Ikizler, 7 Edward D. Siew, 7, 8 Lorraine B. Ware, 9 Kathleen D. Liu, 10 Alan S. Go, 10, 11 James S. Kaufman, "Biomarkers of inflammation and repair in kidney disease progression." Clinical Medicine, p. 10, 2021.

References

[22] A. Advani, “Acute Kidney Injury: A Bona Fide Complication of Diabetes,” *Diabetes*, vol. 69, no. 11, pp. 2229–2237, Nov. 2020 .

[23] M. K. Mohammed, M. J. Ewadh, and A. Hamza, “Beta trace protein level as a better diagnostic marker of renal impairment in patients with chronic kidney disease, diabetes mellitus, and renal transplants,” *J. Pharm. Sci. Res.*, vol. 10, no. 6, pp. 1615–1618, 2018.

[24] I. Lousa, F. Reis, I. Beirão, R. Alves, L. Belo, and A. Santos-Silva, “New potential biomarkers for chronic kidney disease management — A review of the literature,” *Int. J. Mol. Sci.*, vol. 22, no. 1, pp. 1–37, Jan. 2021 .

[25] B. Wang, Z.-L. Li, Y.-L. Zhang, Y. Wen, Y.-M. Gao, and B.-C. Liu, “Hypoxia and chronic kidney disease,” *eBioMedicine*, vol. 77, p. 103942, 2022.

[26] E. M. Senan *et al.*, “Diagnosis of Chronic Kidney Disease Using Effective Classification Algorithms and Recursive Feature Elimination Techniques,” *Journal of Healthcare Engineering*, vol. 2021. 2021.

[27] C. P. Kovesdy, “Epidemiology of chronic kidney disease: an update 2022,” *Kidney International supplements* , vol. 12, no. 1, pp. 7–11, 2022.

[28] B. Bikbov, C. A. Purcell, A. S. Levey, M. Smith, and A. Abdoli, “Global, regional, and national burden of chronic kidney disease, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017,” *Lancet*, vol. 395, no. 10225 ,pp.709-733,2020 .

[29] P. Suriyong, C. Ruengorn, C. Shayakul, P. Anantachoti, and P. Kanjanarat, “Prevalence of chronic kidney disease stages 3–5 in low- and middle-income countries in Asia: A systematic review and meta-analysis,” *PLoS One*, vol. 17, no. 2, p. e0264393, 2022.

[30] S. Bouya, A. Balouchi, H. Rafiemanesh, and M. Hesaraki, “Prevalence of chronic kidney disease in Iranian general population: a meta-analysis and systematic review,” *Therapeutic Apheresis and Dialysis*, vol. 22, no. 6, pp. 594–599, 2018.

[31] D. Mousa ,*A.Alharbi , I. Helal.*, “Prevalence and associated factors of chronic kidney disease among relatives of hemodialysis patients in Saudi Arabia,” *Kidney International reports*, vol. 6, no. 3, p. 817, 2021.

[32] S. Nazareth and S. Krishnamoorthy, “Renal Calculus Obstruction with Emphysematous Pyelonephritis in a Diabetic Male: A Lethal Combo,” *Ann Clin Case Rep*, vol. 6, p. 1946, 2021.

[33] P. Khalili,*Z.Jamali ,T.Sadeghi*, “Risk factors of kidney stone disease: a cross-sectional study in the southeast of Iran,” *BMC Urology*, vol. 21, no. 1. 2021.

[34] X. Tang and J. C. Lieske, “Acute and chronic kidney injury in nephrolithiasis,” *Current. Opinion in Nephrologyand Hypertension.*, vol. 23, no. 4, p. 385, 2014.

[35] M. Kumar, A. Seth, A. K. Singh, M. S. Rajput, and M. Sikandar, “Remediation strategies for heavy metals contaminated ecosystem: A

review," *Environ. Sustain. Indic.*, vol. 12, p. 100155, 2021.

[36] P. Lentini, L. Zanoli, A. Granata, S. S. Signorelli, P. Castellino, and R. Dell'Aquila, "Kidney and heavy metals-The role of environmental exposure," *Mol. Med. Rep.*, vol. 15, no. 5, pp. 3413–3419, 2017.

[37] T. A. Natoli, V. Modur, and O. Ibraghimov-Beskrovnaya, "Glycosphingolipid metabolism and polycystic kidney disease," *Cellular Signaling*, vol. 69, p. 109526, 2020.

[38] P. Gabow,A. Johnson ,*W.Kaehny* "Factors affecting the progression of renal disease in autosomal-dominant polycystic kidney disease," *Pediatric Nephrology*, vol. 7, no. 2. p. 198, 1993 .

[39] Q. Hu, Y. Chen, T. Bao, and Y. Huang, "Association of metabolic dysfunction-associated fatty liver disease with chronic kidney disease: a Chinese population-based study," *Ren. Fail.*, vol. 44, no. 1, pp. 2006–2015, Dec. 2022 .

[40] D. J. Selig, K. S. Akers, K. K. Chung, K. A. Pruskowski, J. R. Livezey, and E. D. Por, "Meropenem pharmacokinetics in critically ill patients with or without burn treated with or without continuous veno-venous haemofiltration," *British Journal of Pharmacology* , vol. 88, no. 5, pp. 2156–2168, 2022.

[41] D. Jarczak, S. Kluge, and A. Nierhaus, "Sepsis—pathophysiology and therapeutic concepts," *Frontiers in. Medicine*, p. 609, 2021.

[42] I. Arany and R. L. Safirstein, "Cisplatin nephrotoxicity," in *Seminars in nephrology*, 2003, vol. 23, no. 5, pp. 460–464.

[43] J. Wang, H. Xiang, Y. Lu, T. Wu, and G. Ji, "New progress in drugs

treatment of diabetic kidney disease," *Biomedicine and Pharmacotherapy,* vol. 141, p. 111918, 2021.

[44] P. M. Ferraro, M. Bargagli, and Trinchieri, "Risk of kidney stones: influence of dietary factors, dietary patterns, and vegetarian–vegan diets," *Nutrients*, vol. 12, no. 3, p. 779, 2020 .

[45] F. Khan, M. F. Haider, M. K. Singh, P. Sharma, T. Kumar, and E. N. Neda, "A comprehensive review on kidney stones, its diagnosis and treatment with allopathic and ayurvedic medicines," *Urol Nephrol Open Access J,* vol. 7, no. 4, pp. 69–74, 2019.

[46] A. Bello ,M . Alrukhaimi , G. Ashuntantang *et al.*, "Complications of chronic kidney disease: current state, knowledge gaps, and strategy for action," *Kidney International Supplements,* vol. 7, no. 2, pp. 122–129, 2017.

[47] S. Soares , R. Vitorino, "Reactivity of human salivary proteins families toward food polyphenols," *Journal of Agricultural and Food Chemistry Food,* vol. 59, no. 10, pp. 5535–5547, 2011.

[48] S. Kalasin, P. Sangnuang, P. Khownarumit, I. M. Tang, and W. Surareungchai, "Salivary creatinine detection using a Cu (I)/Cu (II) catalyst layer of a supercapacitive hybrid sensor: a wireless IoT device to monitor kidney diseases for remote medical mobility," *ACS Biomaterials Science and Engineering,* vol. 6, no. 10, pp. 5895–5910, 2020.

[49] G. Setti,ME Pezzi , MV Viani, TA Pertinhez "Salivary microRNA for diagnosis of cancer and systemic diseases: a systematic review," *International Journal of Molecular Sciences,* vol. 21, no. 3, p. 907,

2020.

[50] G. Ponti, M. Manfredini, and A. Tomasi, “Non-blood sources of cell-free DNA for cancer molecular profiling in clinical pathology and oncology,” *Critical Reviews in Oncology / Hematology*, vol. 141, pp. 36–42, 2019.

[51] Z. Huang ,X Yang, Y Huang, Z Tang, Y Chen , “Saliva–a new opportunity for fluid biopsy,” *Clinical Chemistry and Laboratory Medicine*, vol. 61, no. 1, pp. 4–32, 2023.

[52] D. Lin SW Yang, CL Hsieh, KJ Hsu, T Gong, “Tandem quantification of multiple carbohydrates in saliva using surface-enhanced Raman spectroscopy,” *ACS sensors*, vol. 6, no. 3, pp. 1240–1247, 2021.

[53] I. C. C. Ferreira ,EMG Aguiar, ATF Silva, LLD Santos “Attenuated total reflection-fourier transform infrared (ATR-FTIR) spectroscopy analysis of saliva for breast cancer diagnosis,” *J. Oncol.*, vol. 2020, 2020.

[54] H. J. Kim , TE Kim, M Han, Y Yi, JC Jeong, HJ Chin “Effects of blood urea nitrogen independent of the estimated glomerular filtration rate on the development of anemia in non-dialysis chronic kidney disease: The results of the KNOW-CKD study,” *PLoS ONE*, vol. 16, no. 9 September. 2021.

[55] P. A. Gallardo and C. P. Vio, “Glomerular Filtration and Renal Blood Flow,” in *Renal Physiology and Hydrosaline Metabolism*, Springer, pp. 29–51 , 2022.

[56] “Changing serum creatinine in the detection of acute renal failure and

recovery following radiocontrast studies among acutely ill inpatients Reviewing insights regarding renal functional reserve gained by largedata analysis." Yuri Gorelik a,1, Zaid Abassi b,1, Natalie Bloch-Isenberg a, Mogher Khamaisi a, Samuel N. Heyman c,*, p. 6, 2022.

[57] L. A. Inker, ND Eneanya ,J C oresh "New creatinine-and cystatin C–based equations to estimate GFR without race," *New England Journal of Medicine*, vol. 385, no. 19, pp. 1737–1749, 2021.

[58] S. Chen and R. Chiaramonte, "Estimating creatinine clearance in the nonsteady state: The determination and role of the true average creatinine concentration," *Kidney Med.*, vol. 1, no. 4, pp. 207–216, 2019.

[59] Lesley A. Inker1 Kidney Disease, Race, and GFR Estimation Andrew S. Levey, 1 Silvia M. Titan, 1 Neil R. Powe, 2 Josef Coresh, "Kidney Disease Raceand GFR Estimation." Journal of American Society of Nephrology, p. 13, 2020.

[60] S. M. Figueroa, P. Araos, J. Reyes, B. Gravez, J. Barrera-Chimal, and C. A. Amador, "Oxidized albumin as a mediator of kidney disease," *Antioxidants*, vol. 10, no. 3. pp. 1–13, 2021.

[61] A. Tojo, "Mechanism underlying selective albuminuria in minimal change Nephrotic syndrome," *International Journal of Nephrology*, vol. 2019, 2019.

[62] H. Wang, S. Huang, T. Hu, S. Fei, and H. Zhang, "Circ_0000064 promotes high glucose-induced renal tubular epithelial cells injury to facilitate diabetic nephropathy progression through miR-532-3p/ROCK1 axis," *BMC Endocrine Disorder*, vol. 22, no. 1, pp. 1–14,

2022.

[63] L. Yu ,Y Wang, YH Guo, L Wang and Z Yang,"HIF-1α Alleviates High-Glucose-Induced Renal Tubular Cell Injury by Promoting Parkin/PINK1-Mediated Mitophagy," *Front. Med.*, vol. 8, p. 2875, 2022.

[64] T. A. Ikizler, "KDOQI clinical practice guideline for nutrition in CKD: 2020 update," *Amrican Journal of Kidney Diseases..*, vol. 76, no. 3, pp. S1–S107, 2020.

[65] S. Chung, S. Kim, and Son, "Empagliflozin contributes to polyuria via regulation of sodium transporters and water channels in diabetic rat kidneys," *Front. Physiol.*, vol. 10, p. 271, 2019.

[66] S. A. G. Lava, C. Zollinger, H. Chehade, D. Schaffner, N. Sekarski, and S. Di Bernardo, "Diuretics in pediatrics," *Euopean Journal of P ediatricsr*, pp. 1–12, 2023.

[67] K. Namekawa, M. Tokoro Schreiber, T. Aoyagi, and M. Ebara, "Fabrication of zeolite-polymer composite nanofibers for removal of uremic toxins from kidney failure patients," *Biomaterials Science*, vol. 2, no. 5. pp. 674–679, 2014.

[68] K. E. Manera, D. W. Johnson, and Craig, "Establishing a core outcome set for peritoneal dialysis: report of the SONG-PD (Standardized Outcomes in Nephrology–Peritoneal Dialysis) consensus workshop," *Am. J. Kidney Dis.*, vol. 75, no. 3, pp. 404–412, 2020.

[69] J. Thomas, "Application of Artificial Intelligence in Thyroidology,"

Artificial Intelligence. pp. 273–283, 2020.

[70] I. Lousa, F. Reis, I. Beirão, R. Alves, L. Belo, and A. Santos-Silva, "New potential biomarkers for chronic kidney disease management—a review of the literature," *International Journal of Molecular Sciences*, vol. 22, no. 1, p. 43, 2020.

[71] J. Wajda, P. Dumnicka, and M.Sporek, "Does Beta-Trace Protein (BTP) outperform cystatin C as a diagnostic marker of acute kidney injury complicating the early phase of acute pancreatitis?," *Journal Clinical Medicine,* vol. 9, no. 1, p. 205, 2020.

[72] E. Orenes-Piñero, S. Manzano-Fernández, Á. López-Cuenca, F. Marín, M. Valdés, and J. L. Januzzi, "β-Trace protein: from GFR marker to cardiovascular risk predictor," *Clinical Journalof American Society of Nephrology*, vol. 8, no. 5, pp. 873–881, 2013.

[73] S. Schwab, D. Pörner, and Kleine, "Pre-transplant serum Beta Trace Protein indicates risk for post-transplant major cardiac adverse events," *Nephrology*, vol. 28, no. 1, pp. 51–59, 2023.

[74] L. A. Obert, S. A. Elmore, D. Ennulat, and K. S. Frazier, "A Review of Specific Biomarkers of Chronic Renal Injury and Their Potential Application in Nonclinical Safety Assessment Studies," *Toxicologic Pathology*, vol. 49, no. 5. pp. 996–1023, 2021.

[75] C. A. White, S. Ghazan-Shahi, and M. A. Adams, "β-Trace protein: a marker of GFR and other biological pathways," *American Journal of Kidney Diseases*, vol. 65, no. 1, pp. 131–146, 2015.

[76] E. Iversen, L. Boesby, D. Hansen, and M. B. Houlind, "Comparison

of 24-hour urinary creatinine clearance and estimated glomerular filtration rate based on a panel of filtration markers in patients with chronic kidney disease," *Pharmacology Research and Perspectives*, vol. 10, no. 5, p. e01002, 2022.

[77] S. Shimamoto,*Y. Nakagawa ,and Y.Hidaka*, "Ligand recognition of lipocalin-type prostaglandin D synthase," *Biophys. J.*, vol. 102, no. 3, p. 252a, 2012.

[78] Y. Urade, "Biochemical and Structural Characteristics, Gene Regulation, Physiological, Pathological and Clinical Features of Lipocalin-Type Prostaglandin D2 Synthase as a Multifunctional Lipocalin," *Frontiers in Physiology*, vol. 12. 2021.

[79] W. L. Smith, Y. Urade, and P.-J. Jakobsson, "Enzymes of the cyclooxygenase pathways of prostanoid biosynthesis," *Chemical Reviews*, vol. 111, no. 10, pp. 5821–5865, 2011.

[80] M. A. Gallant, R. Samadfam, J. A. Hackett, J. Antoniou, J. Parent, and A. J. de Brum-Fernandes, "Production of prostaglandin D2 by human osteoblasts and modulation of osteoprotegerin, RANKL, and cellular migration by DP and CRTH2 receptors," *Journal of Bone and Mineral Research*, vol. 20, no. 4, pp. 672–681, 2005.

[81] A. Szymczak, M. Kusztal, and T.Gołębiowski, "High Plasma Angiopoietin-2 Levels Predict the Need to Initiate Dialysis within Two Years in Patients with Chronic Kidney Disease," *International Journal of MolecularSciences*, vol. 24, no. 12, p. 10036, 2023.

[82] C. A. White, A.Akbari and S.Doucette"Effect of clinical variables and immunosuppression on serum cystatin C and beta-trace protein in

kidney transplant recipients," *American Journal of kidney Diseases*, vol. 54, no. 5, pp. 922–930, 2009.

[83] K. B. Shanmugasundaram, J. Li, A. A. I. Sina, A. Wuethrich, and M. Trau, "Toward precision oncology: SERS microfluidic systems for multiplex biomarker analysis in liquid biopsy," *Materials Advances*, 2022.

[84] M. M. A. L. Pelsers, A. Morovat, G. J. M. Alexander, W. T. Hermens, A. K. Trull, and J. F. C. Glatz, "Liver fatty acid-binding protein as a sensitive serum marker of acute hepatocellular damage in liver transplant recipients," *Clinical Chemistry*, vol. 48, no. 11, pp. 2055–2057, 2002.

[85] M. Yanishi and H. Kinoshita, "Urinary L-type fatty acid-binding protein is a predictor of cisplatin-induced acute kidney injury," *BMC Nephrology.*, vol. 23, no. 1, pp. 1–6, 2022.

[86] Y. He *et al.*, "A nuclear magnetic resonance-based structural rationale for contrasting stoichiometry and ligand binding site (s) in fatty acid-binding proteins," *Biochemistry*, vol. 50, no. 8, pp. 1283–1295, 2011.

[87] T. Sunayama *et al.*, "Urinary liver-type fatty acid-binding protein as a prognostic marker in patients with acute heart failure," *ESC Hear. Fail.*, vol. 9, no. 1, pp. 442–449, 2022.

[88] R. Wang, C. Yao, and F. Liu, "Association between renal podocalyxin expression and renal dysfunction in patients with diabetic nephropathy: a single-center, retrospective case-control study," *Biomed Res. Int.*, vol. 2020, 2020.

[89] M. S. Younes-Ibrahim and M. Younes-Ibrahim, "Biomarkers and kidney diseases: a brief narrative review," *Journal of Laboratory and Precision Medicine*, vol. 7. 2022.

[90] N. M. Panduru ,W.Fu ,D.Wang and R. Deng., "Urinary liver-type fatty acid–binding protein and progression of diabetic nephropathy in type 1 diabetes," *Diabetes Care*, vol. 36, no. 7, pp. 2077–2083, 2013.

[91] D. M. Green , "Long-term renal function after treatment for unilateral, nonsyndromic Wilms tumor. A report from the St. Jude Lifetime Cohort Study," *Pediatr. Blood Cancer*, vol. 67, no. 10, p. e28271, 2020.

[92] D. Mousa A. Alharbi and I. Helal, "Prevalence and Associated Factors of Chronic Kidney Disease among Relatives of Hemodialysis Patients in Saudi Arabia," *Kidney International Reports*, vol. 6, no. 3. pp. 817–820, 2021.

[93] A. O. Onyemelukwe, "Impact of Alcohol Intoxication on Pregnant Albino Wistar Rats; Some Blood Profile and Histopathological Evidence," *Annu. Res. Rev. Biol.*, pp. 7–16, 2020.

[94] J. H. Bernard, *Clinical diagnosis and managment by laboratory methods*. Saunders, 1996.

[95] M. Khorashadi, R. Beunders, P. Pickkers, and M. Legrand, "Proenkephalin: a new biomarker for glomerular filtration rate and acute kidney injury," *Nephron*, vol. 144, no. 12, pp. 655–661, 2020.

[96] P. Asia, A. Sharma, A. Kumar Ahirwar, S. Garg, J. Elgiva John, and N. Gopal, "The study of ischemia modified albumin as an early biomarker of epilepsy in adolescent population: a cross-sectional

study," *Horm. Molecular Biology and Clinical Investigation Clin.* vol. 42, no. 2, pp. 183–187, 2021.

[97] C. A. Burtis, *Tietz textbook of clinical chemistry*. Saunders, 1999.

[98] M. C. de M. S. Coelho, L. B. Leal, M. S. Nogueira, P. A. A. Castro, F. Peralta, and D. M. Zezell, "Biochemical characterization of saliva of smoking and non-smoking patients by using Fourier-transform infrared spectroscopy," in *Biomedical Vibrational Spectroscopy 2022: Advances in Research and Industry*, vol. 11957, pp. 86–97 ,2022 .

[99] A. J. Mahrath, S. M. Selman, and S. S. Hammoud, "Use of alhagi roots extract as new alternative source of nutrition Part II," in *Journal of Physics: Conference Series*, vol. 1294, no. 6, p. 62026 , 2019 .

[100] U. Branavan, K. Muneeswaran, W. S. S. Wijesundera, A. Senanayake, N. V. Chandrasekharan, and C. N. Wijeyaratne, "Association of Kiss1 and GPR54 Gene Polymorphisms with Polycystic Ovary Syndrome among Sri Lankan Women," *Biomed Research International* , vol. 2019, pp. 1–10, Mar. 2019 .

[101] A. Al-Aaraji, S. Al-Qaysi, and A. Baay, "Role of periostin in Iraqi asthmatic patients," *Medical Journal of Babylon*, vol. 16, no. 3, p. 256, 2019 .

[102] M. Jhamb , "Association of sleep apnea with mortality in patients with advanced kidney disease," *Clinical Journal of American Soc. Nephrol.*, vol. 15, no. 2, pp. 182–190, 2020.

[103] R. Simon, R. Snow, and S. Wakeman, "Understanding why patients with substance use disorders leave the hospital against medical

advice: a qualitative study," *Substance Abuse.*, vol. 41, no. 4, pp. 519–525, 2020.

[104] N. Amin, R. T. Mahmood, and MJ Asad, "Evaluating urea and creatinine levels in chronic renal failure pre and post dialysis: a prospective study," *J. Cardiovasc. Dis.*, vol. 2, no. 2, pp. 1–4, 2014.

[105] A. Devagourou and K. K. Sharma, "An experimental study to evaluate the effect of low-intensity intradialytic exercises on serum urea, creatinine, and fatigue of chronic kidney disease patients undergoing hemodialysis," *Saudi Journal of Kidney Diseases and Transplantation* , vol. 32, no. 5, pp. 1253–1259, 2021.

[106] نجم. م. العوكلي, ن. and الصويعي, "Concentrations of Serum creatinine in blood of patients visiting Tamimi, Al baida and Alabyar Hospitals," *مجلة المنارة العلمية*, no. 1, 2020.

[107] C. J. Janmaat*M Van Diepen ,A Gaspariri and M Evans*, "Lower serum calcium is independently associated with CKD progression," *Scientific Reports* , vol. 8, no. 1, pp. 1–9, 2018.

[108] J. Wang, L. Zhang, Z. Yang, J. Liu, and W. Guo, "Related factors and economic burden evaluation of nosocomial infection in patients with chronic kidney disease," *Contrast Media Mol. Imaging*, vol. 2022, 2022.

[109] L. Szablewski, "Distribution of glucose transporters in renal diseases," *Journal of Biomedical Science*, vol. 24, no. 1. 2017.

[110] B. Yao, M.-C. Giel, and Y. Hong, "Detection of kidney disease biomarkers based on fluorescence technology," *Mater. Chem. Front.*,

vol. 5, no. 5, pp. 2124–2142, 2021.

[111] L. A. Inker ,SJ Couture, H Tighiouart, AG_Abraham ,"A new panel-estimated GFR, including β2-microglobulin and β-trace protein and not including race, developed in a diverse population," *American Journal of Kidney Dieases* ,vol. 77, no. 5, pp. 673–683, 2021.

[112] M. C. Foster , FW.Dekker, RT Krediet and AL Simon, "Non-GFR determinants of low-molecular-weight serum protein filtration markers in the elderly: AGES-Kidney and MESA-Kidney," *American Journal of Kidney Diseases*, vol. 70, no. 3, pp. 406–414, 2017.

[113] M. Katayama, T. Miyazaki, and K. Ohata, "Temporal changes in urinary excretion of liver-type fatty acid binding protein (L-FABP) in acute kidney injury model of domestic cats: a preliminary study," Journal of Veterinary *Medical Science* , vol. 81, no. 12, pp. 1868–1872, 2019.

[114] A. Kamijo-Ikemori, T. Sugaya, T. Yasuda, T. Kawata, and Ota, "Clinical Significance of Urinary Liver-Type Fatty Acid–Binding Protein in Diabetic Nephropathy of Type 2 Diabetic Patients," *Diabetes Care*, vol. 34, no. 3, pp. 691–696, Mar. 2011 .

[115] T. N. D. Thi, B. N. Gia, H. L. Le Thi, T. N. C. Thi, and H. P. Thanh, "Evaluation of urinary L-FABP as an early marker for diabetic nephropathy in type 2 diabetic patients," *Journal of Medical biochemistry*, vol. 39, no. 2, p. 224, 2020.

[116] A. Vijayan ,"Clinical use of the urine biomarker [TIMP-2]×[IGFBP7] for acute kidney injury risk assessment," *American Journal of Kidney Diseases* , vol. 68, no. 1, pp. 19–28, 2016.

[117] A. Kamijo-Ikemori, T. Sugaya, D. Ichikawa, and Hoshino, "Urinary liver type fatty acid binding protein in diabetic nephropathy," *Clinica ChimicaActa*, vol. 424, pp. 104–108, Sep. 2013 .

[118] A. Gluba-Brzózka, B. Franczyk, R. Olszewski, and J. Rysz, "The influence of inflammation on anemia in CKD patients," *International Journal of Molecular Sciences* ,vol. 21, no. 3, p. 725, 2020.

[119] H. Goel, J. Melot, M. D. Krinock, A. Kumar, S. K. Nadar, and G. Y. H. Lip, "Heart-type fatty acid-binding protein: An overlooked cardiac biomarker," *Annals of Medicine* , vol. 52, no. 8, pp. 444–461, 2020.

[120] Y. P. See ,*H. Htay and A.Teixeira* "Utility of serum beta-trace protein as a tool for estimating residual kidney function in peritoneal dialysis patients," *Perit. Dial. Int.*, vol. 41, no. 2, pp. 226–235, 2021.

[121] M. Kobata, A Shimizu and H Rinno "β-trace protein, a new marker of GFR, may predict the early prognostic stages of patients with type 2 diabetic nephropathy," *Journal of Clinical Laboratory Analysis*, vol. 18, no. 4, pp. 237–239, 2004.

[122] B. Campanella, S. Legnaioli, M. Onor, E. Benedetti, and E . Bramanti, "The Role of the Preanalytical Step for Human Saliva Analysis via Vibrational Spectroscopy," *Metabolites*, vol. 13, no. 3, p. 393, 2023.

[123] G. Cakmak-Arslan, H. Haksoy, P. Goc-Rasgele, and M. Kekecoglu, "Determination of the dose-dependent toxic effects of mad honey on mouse liver using ATR-FTIR spectroscopy," *Spectrochimica Acta-Part A:Molecular and Biomolecular Spectroscopy*, vol. 228, p. 117719, 2020.

[124] R. P. C. B. Rodrigues *et al.*, "Differential molecular signature of human saliva using ATR-FTIR spectroscopy for chronic kidney disease diagnosis," *Brazilian Dental Journal* , vol. 30, pp. 437–445, 2019.

[125] M. Maciejczyk, K. Taranta-Janusz, A. Wasilewska, A. Kossakowska, and A. Zalewska, "A case-control study of salivary redox homeostasis in hypertensive children. Can salivary uric acid be a marker of hypertension?," *Journal of Clinical Medicine* , vol. 9, no. 3, p. 837, 2020.

[126] S. Khaustova, M. Shkurnikov, E. Tonevitsky, V. Artyushenko, and A. Tonevitsky, "Noninvasive biochemical monitoring of physiological stress by Fourier transform infrared saliva spectroscopy," *Analyst*, The vol. 135, no. 12, pp. 3183–3192, 2010.

[127] M. Dajak, S. Ignjatović, B. Stojimirović, S. Gajić, and N. Majkić-Singh, "Beta-trace protein as a marker of renal dysfunction in patients with chronic kidney disease: comparison with other renal markers," *Journal of Medical Biochemistry*, vol. 29, no. 2, pp. 66–72, 2010.

[128] L. A. Obert, S. A. Elmore, D. Ennulat, and K. S. Frazier, "A review of specific biomarkers of chronic renal injury and their potential application in nonclinical safety assessment studies," *Toxicologic Pathology*, vol. 49, no. 5, pp. 996–1023, 2021.

[129] U. Pöge, T Gerhardt and B Stoffel-Wagner "Beta-trace protein-based equations for calculation of GFR in renal transplant recipients," *American Journal of Transplantation*, vol. 8, no. 3. pp. 608–615, 2008.

[130] J. Wajda *et al.*, "Does Beta-Trace Protein (BTP) outperform cystatin C as a diagnostic marker of acute kidney injury complicating the early phase of acute pancreatitis?," *Journal of Clinical Medicine*, vol. 9, no. 1, p. 205, 2020.

[131] K. S. Spanaus, B. Kollerits, E. Ritz, M. Hersberger, F. Kronenberg, and A. Von Eckardstein, "Serum creatinine, cystatin C, and β-trace protein in diagnostic staging and predicting progression of primary nondiabetic chronic kidney disease," *Clinical Chemistry*, vol. 56, no. 5. pp. 740–749, 2010.

Appendix

Results of (Hydroxyl and Amide) Bands by Using Attenuated Total Reflection-Fourier Transform Infrared (ATR-FTIR) Spectroscopy

N	Group	Hydroxyl	Amide
1	CKD	3334	1635
2	CKD	3293	1634
3	CKD	3312	1635
4	CKD	3324	1635
5	CKD	3333	1637
6	CKD	3309	1636
7	CKD	3337	1640
8	CKD	3286	1636
9	CKD	3327	1636
10	CKD	3329	1638
11	CKD	3359	1641
12	CKD	3342	1640
13	CKD	3378	1645
14	CKD	3302	1649
15	CKD	3387	1642
16	CKD	3373	1644
17	CKD	3373	1642
18	CKD	3383	1642
19	CKD	3358	1643
20	AKI	3376	1639
21	AKI	3387	1644
22	AKI	3385	1644
23	AKI	3384	1642
24	AKI	3386	1642
25	AKI	3383	1645
26	AKI	3375	1640
27	AKI	3381	1638
28	AKI	3381	1642
29	AKI	3387	1644
30	AKI	3388	1644
31	AKI	3373	1645
32	AKI	3374	1635
33	AKI	3356	1640
34	AKI	3344	1643
35	AKI	3357	1643

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36	AKI	3374	1643
37	AKI	3380	1644
38	AKI	3331	1639
39	Control	3385	1636
40	Control	3385	1647
41	Control	3385	1637
42	Control	3382	1642
43	Control	3412	1643
44	Control	3377	1639
45	Control	3387	1643
46	Control	3394	1640
47	Control	3383	1638
48	Control	3388	1643
49	Control	3385	1642
50	Control	3396	1642
51	Control	3384	1644
52	Control	3375	1640
53	Control	3387	1644
54	Control	3359	1643
55	Control	3405	1643
56	Control	3385	1644
57	Control	3386	1644
58	Control	3401	1647
59	Control	3377	1639
60	Control	3388	1644
61	Control	3385	1644
62	Control	3356	1642
63	Control	3379	1643
64	Control	3400	1645
65	Control	3379	1642
66	Control	3385	1643
67	Control	3374	1639
68	Control	3391	1643
69	Control	3384	1643
70	Control	3381	1640
71	Control	3386	1640
72	Control	3379	1644
73	Control	3376	1641

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74	Control	3376	1640
75	Control	3373	1638
76	Control	3362	1643
77	Control	3375	1640
78	Control	3389	1645
79	Control	3385	1639
80	Control	3376	1639

الخلاصة

مرض الكلى (KD) هو فقدان وظائف الكلى بشكل تدريجي ونهاني أو تلف كلوي مستمر ناتج عن العديد من العوامل الأساسية مثل مرض السكري وارتفاع ضغط الدم. يمكن تصنيفه بناءً على سبب وشدة التشوهات التركيبية والوظيفية ومدة تلك التشوهات. هدفت الدراسة لتقييم دور بروتين (BTP) وبروتين (LFABP) كمؤشرات حديثة لمرض الكلى وتم قياسها باستخدام تقنية الامتزاز المناعي الانزيمي (ELISA). وكذلك ، لفحص المكونات اللعابية المتمثلة بحزم الاهتزار (هيدروكسيل وأميد) كمؤشراولي لبدء الفشل الكلوي باستخدام تقنية مطيافية الاشعة تحت الحمراء ومنظومة الانعكاس الكلي (ATR-FTIR) .

اشتملت الدراسة الكلية على 107 عينة ،وبعد استبعاد (27) عينة لعيوب تحليلية خاصة لعينات اللعاب. تبقى 80 مشاركًا تتراوح أعمارهم بين 25-63 عامًا , ومؤشر كتلة الجسم (BMI) 19-29 كجم / م^2. تم تقسيم هؤلاء المشاركين إلى 38 مريضًا بأمراض الكلى (19 مصابًا بإصابة كلوية حادة و 19 مصابًا بمرض كلوي مزمن) و 42 شخص سليم . حيث تم تقدير مستويات مصل الدم من (الجلوكوز واليوريا والكرياتينين والألبومين) بطرق المطياف اللوني (UV-Vis) ، والكالسيوم الكلي والمؤين باستخدام تقنية تحليل الالكتروليتات. وكذلك تم قياس بروتين(BTP) وبروتين (LFABP) في المصل مع حزم الاهتزازات للهيدروكسيل والامايد في اللعاب .

أظهرت نتائج الدراسة زيادة معنوية ($p < 0.05$) في اليوريا والكرياتينين في مرضى الكلى عند مقارنتها الاصحاء ، بينما ظهرمعدل الترشيح الكبيبي (GFR) ،) و الكالسيوم الكلي والمؤين انخفاضًا معنويًا ($p < 0.05$). في نفس المقارنة مع تغيرات معنوية ($p < 0.05$) في مستوى الجلوكوز. من ناحية أخرى ، زيادة معنوية لبروتين (BTP) ($p < 0.05$) في المجموعة لأمراض الكلى المزمنة (CKD) بالمقارنة مع مجموعة إصابة الكلى الحادة (AKI) ومجموعه الاصحاء حيث كان متوسط الفرق (21.974، 20.814) على التوالي , بينما اظهر بروتين (LFABP) انخفاضا معنويا (قيمة $p < 0.05$) في مرضى الكلى الحادة (AKI) بالمقارنة مع مجموعة مرضى الكلى المزمنة (AKD) والمجموعة الاصحاء (21.222، 15.306-) على التوالي. كذلك اظهرت حزم الهيدروكسيل والامايد اللعابي انخفاضا معنويا ملحوظ بين مرضى إصابات الكلى الحادة (AKI) ومجموعة الاصحاء.

أظهر تحليل المقارن بين مرضى الكلى ارتباطًا إيجابيًا بين بروتين (BTP) وبروتين(LFABP) كما في قيمة ($r = 0.815$، $p = 0.000$) ، بينما لوحظ ارتباط سلبي لمعدل ترشيح الكبيبات (GRR) مع بروتين (BTP) وبروتين (LFABP) ($r = 0.414$، $P = 0.010$) ، ($r = 0.350$، $p = 0.036$)على التوالي. علاوة على ذلك تم تسجيل ارتباط إيجابي بين حزم الهيدروكسيل والاميد اللعابي ($r = 0.564$، $p = 0.000$). وكذلك اظهرت نتيجة تحليل ROC نتيجة قوية للغاية (91.1%) لحزمة الهيدروكسيل اللعابية في مرضى أمراض الكلى المزمنة (CKD) مقارنة بمجموعة الاصحاء ، $P = 0.000$ ، بحزمة قطع يساوي (3373.20 سم$^{-1}$). وبالمثل ، كان

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قيمة(ROC) لحزمة الهيدروكسيل اللعابي لمرضى أمراض الكلى المزمنة (CKD) اكثر من إصابة الكلى الحادة (AKI)بقيمة 83.41٪ ، P = 0.000 عند حزمة القطع تساوي (3343 سم$^{-1}$).

تشير نتائج هذه الدراسة إلى أن البروتين (LFABP) يمكن اعتباره علامة محتملة لتطور الفشل الكلوي ، بينما أظهر بروتين (BTP) حساسية أقل وقد يتطلب مزيدًا من البحث والتحقيق. بالإضافة إلى ذلك ، اظهرت حزم الهيدروكسيل اللعابية مؤشرات حساسة للغاية كدليل لبدء الفشل الكلوي. لذلك تسلط هذه النتائج الضوء على الفائدة المحتملة لهذه الحزم في تشخيص ومراقبة تطورأمراض الكلى .

Chapter one

Introduction

And

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Chapter Two

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Results

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