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***Theme*:** Screening of prediabetes and chronic kidney disease among youth

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Abstract

Chronic kidney disease (CKD) is an alteration in renal function, one of its main causes is diabetes essentially type 2 diabetes (T2D). Evaluation of kidney's function is made through the estimation of the glomerular filtration rate (GFR) that should be over 60ml/min/1.73m2.

Seen that CKD and T2D are a silent diseases, we aim by this research to sensitize youth about the seriousness of these diseases and the importance of the early diagnosis

This present study is based on the investigation of 100 persons aged between 18 and 35 years. A questionnaire has been filled out, followed by a dosage of fasting blood glucose, blood creatinine and urea, urine chemistry test then the determination of the creatinine clearance using MDRD equation.

Our results indicate that a considerable part of the population studied is likely to develop CKD or T2D because of various risk factors involved such us obesity, medications, nutritional habits...etc

Key words: Chronic kidney disease, prediabetes, type 2 diabetes, glomerular filtration rate, fasting blood glucose, blood creatinine, creatinine clearance.

Résumé

La maladie rénale chronique (MRC) est une atteinte rénale dont une de ses causes majeures est le diabète essentiellement le diabète type 2 (DT2). L'évaluation de la fonction rénale se fait par l'estimation du taux de filtration glomérulaire (TFG) dont la valeur normale est moins que 60ml/min/1.73m2. Vu que la MRC et le DT2 sont des maladies silencieuses, notre objectif de ce travail de recherche est de rechercher les malades silencieux et de sensibiliser les jeunes sur la gravité de ces deux maladies ainsi que l'importance du diagnostic précoce.

La présente étude est basée sur une investigation de 100 personnes dont leurs âges varient entre 18 et 35 ans. Un questionnaire a été rempli suivie par un dosage de la glycémie à jeun, créatinine sanguine, urée sanguine, test de chimie des urines et calcul de la clairance de créatinine en utilisant l'équation MDRD.

Nos résultats montrent qu'une partie considérable de la population étudiée est susceptible de développer une MRC ou DT2 en raison de divers facteurs de risque impliqués tels que l'obésité, la prise de certains médicaments, les habitudes alimentaires...etc.

Mots clés : Maladie rénale chronique, pré-diabète, taux de filtration glomérulaire, diabète type 2, glycémie à jeun, créatinine sanguine, clairance de créatinine.

ملخـــــص

مرض الكلى المزمن هواضطراب يصيب الكلى حيث يعد مرض السكري أحد أسبابه الرئيسية بالأخص النوع الثاني. يتم تقييم الوظيفة الكلوية عن طريق تقدير معدل الترشيح الكبيبي والذي تكون قيمته الطبيعية أكبر من 60 مل/دقيقة/ 1.73م^{2}.

نظرًا أن مرض الكلى المزمن و السكري من النوع الثاني هي عبارة عن أمراض تتطور بطريقة صامتة، فإن هدفنا من هذا الأساسي من هذا البحث هو زيادة الوعي بين الشباب حول خطورة هذين المرضين بالإضافة إلى أهمية التشخيص المبكر.

. هذه الدراسة هي عبارة عن بحث يضم 100 شخص تتراوح أعمارهم بين 18 و 35 عامًا. تعتمد أساسا على استبيان يتم الاجابة عليه، متبوعًا بتحليل سكر الدم في حالة الصيام،تحليل كرياتينين الدم ، تحليل يوريا الدم واختبار كيمياء البول بالاضافة الى حساب نسبة تصفية الكرياتينين باستخدام معادلة MDRD.

أظهرت النتائج أن جزءًا كبيرًا من مجموعة لأشخاص الذين تمت عليهم الدراسة أنهم عرضة للاصابة بمرض الكلى المزمن أو السكري من النوع الثاني، وذلك بسبب جملة من العوامل المختلفة على غرار السمنة ، تناول بعض الأدوية ، والعادات الغذائية ، وما إلى ذلك.

الكلمات المفتاحية: مرض الكلى المزمن، معدل الترشيح لكبيبي ، السكري من النوع الثاني، تحليل سكر الدم في حالة صيام، كرياتينين الدم ، يوريا الدم، اختبار كيمياء البول ، نسبة تصفية الكرياتينين

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List of abbreviations

4-AP: 4-Aminophenazone

ACE: Angiotensin-I-converting enzyme

AKI: Acute kidney injury

AT1R: Angiotensin II receptor type 1

AT2R: Angiotensin II receptor type 2

ADA: American diabetes Association

BFU-Es: Burst Forming Unit E

BMI: Body mass index

BUN: Blood urea nitrogen

CFU-Es: Colony Forming Unit E

CKD: Chronic kidney disease

CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration equation

CRI: Chronic renal insufficiency

CrCl : Creatinine clearance rate

CVD: Cardiovascular disease

Cys C: Cystatin C

DKD : Diabetic kidney disease

DM: Diabetes mellitus

ECRI: End chronic renal insufficiency

EPO: Erythropoietin

EPO-R: Erythropoietin- Receptor

GBM: glomerular basement membrane

GFR: Glomerular filtration rate

GOD: Glucose oxidase

HbA1c: Hemoglobin A1C

HDL: High-density lipoprotein

Hcy: Homocysteine

HHcy: Hyperhomocysteinemia

HTA: Arterial hypertension

IFG: Impaired fasting glucose

IGT: Impaired glucose tolerance

LDL: Low-density lipoprotein

MAPK3: Mitogen-activated protein kinase 3

MDRD: Modification of Diet in Renal Disease equation

miRNAs: microRNAs

MRA: Mineralocorticoid receptor antagonist

NSAIDs:Nonsteroidal anti-inflammatory drug

POd: Peroxidase

pri-miRNAs: primary miRNA

pre-miRNAs: precursor

PTEN: Phosphatase and tensin homolog deletions in chromosome

RAS: Renin-angiotensin system

RCTs: Randomized controlled trials

RISC: RNA-induced silencing complex

SGLT2: The Sodium-glucose cotransporter-2 inhibitors

T1D : Type 1 diabetes

T2D : Type 2 diabetes

TGF-beta: Transforming growth factor beta

US: United States

UAER: Urine albumin excretion rate

UACR: Urine albumin-to-creatinine ratio

WHO: World Health Organization

WR: working reagents

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Introduction

Introduction

While public awareness of cardiovascular disease (CVD), obesity, cancer and AIDS can currently be taken for granted, chronic kidney disease (CKD) remains a “silent” epidemic of which diabetes and hypertension are the most common causes (Veeren, 2021). Studies have shown that the kidney is one of the main targets for complications related to diabetes, as a matter of fact, increased blood glucose, or hyperglycaemia, affects the functioning of small blood vessels and the functioning of the kidney: we refer to "kidney complication of diabetes" or "diabetic nephropathy"(Samsu,2021). Furthermore, despite significant improvements since 2001, CKD remains frequent and highly underestimated in patients with type 2 diabetes, knowing that in a late stage, nearly a third of patients (30%), especially those with type 2 diabetes (32%), starting dialysis or kidney transplantation on an emergency basis (Assogba, 2014). These findings suggest that more efforts should be made to educate diabetic patients and doctors on how to better screen.

1. Kidneys organs

The kidneys are the organs that notably ensure the blood filtration through urine production, they are considered as typical purifier, regulator of the body. The kidneys are part of the urinary system which also includes the bladder, two long channels that connect the kidneys with the bladder: the ureters and the urethra (figure 01) (Radi, 2019).

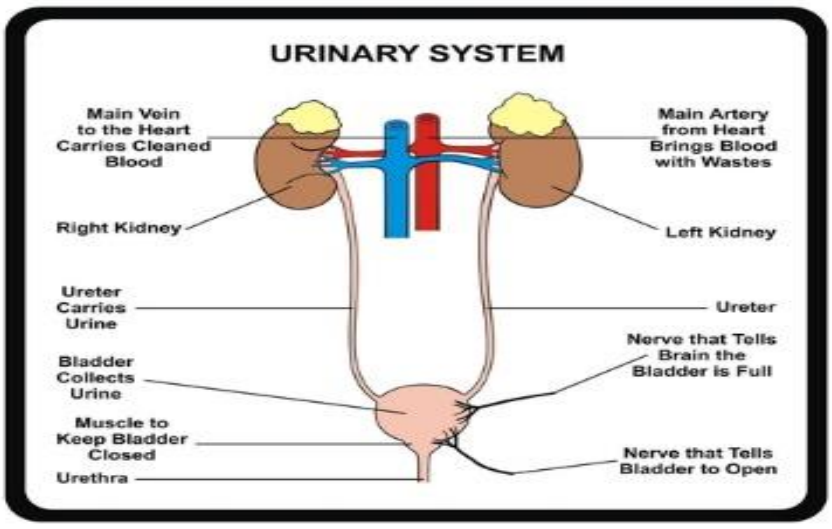

Figure 01: urinary system [01]

1.1. Anatomy of the kidneys organs

1.1.1. Kidney's macroscopic anatomy

Macroscopically, kidneys are organs with a bean shape, each weighing about 100 to 200 grams. They are approximately 12 cm long, 6 cm wide and 5 cm thick [02].They are located behind the peritoneum, in the lumbar fossae on either side of the spine against the posterior abdominal wall at the level of the first lumbar vertebrae and the last two ribs (Figure02). The right kidney is generally a little lower than the left due to the presence of the liver located on the right in the organism (Radi, 2019; Boccara, 2015). Each kidney is surrounded by fibrous, smooth renal capsule, Surrounding the renal capsule is a thick layer of fats to provide protection against trauma. Inside of this we find a structure called the parenchyma which consists of two parts: a peripheral part: the cortex and a central part: the medulla. The medulla is further subdivided into the outer medulla and the inner medulla. The outer medulla is divided into the outer stripe of the outer medulla and the inner stripe of the outer medulla. A set of pyramidal structures called the Malpighian pyramids are also observed within the medulla , their base touches the surface of the kidney which make them covered by the cortex, their apex constitutes the renal papillae. Each papillae goes to the center of the kidney, towards a renal calyx, so that the urine produced in the pyramids flows through the orifices of the papillae located at their apex to reach the minor calyx, the urine produced will accumulate in the major calyx then in the renal pelvis which corresponds to the abutment of the calyxes (or pelvis).

Finally the urine accumulated reaches the bladder through the ureter (figure03). The renal pyramids numbering 8 to 12 per kidney are separated by cortical tissue, this separation forms the renal columns called columns of Bertin, the association of the pyramid with the two columns that surround it forms the renal lobe. (Radi,2019 ; Boccara,2015).

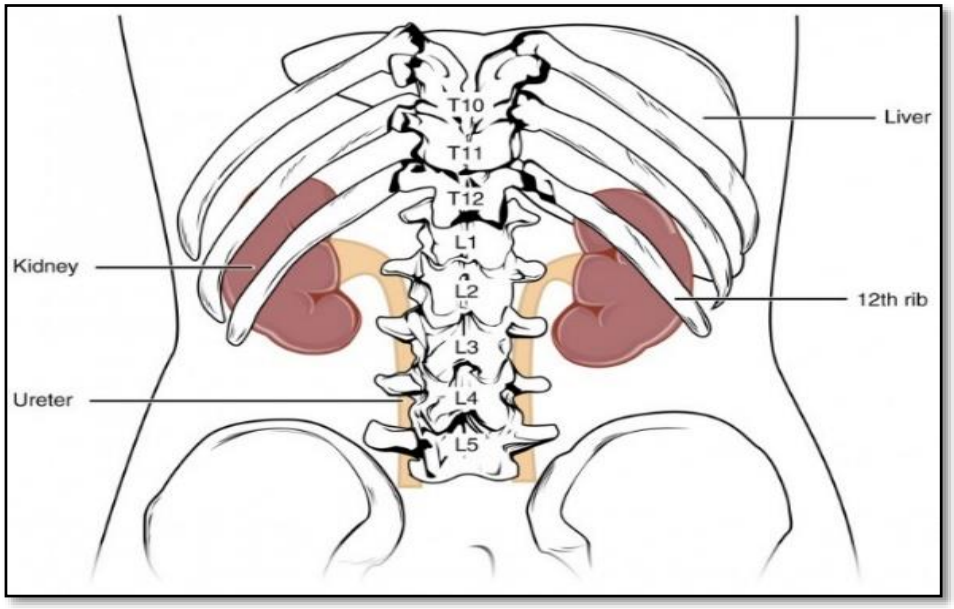

Figure 02: The kidneys are slightly protected by the ribs and are surrounded by fat for protection (not shown) [03]

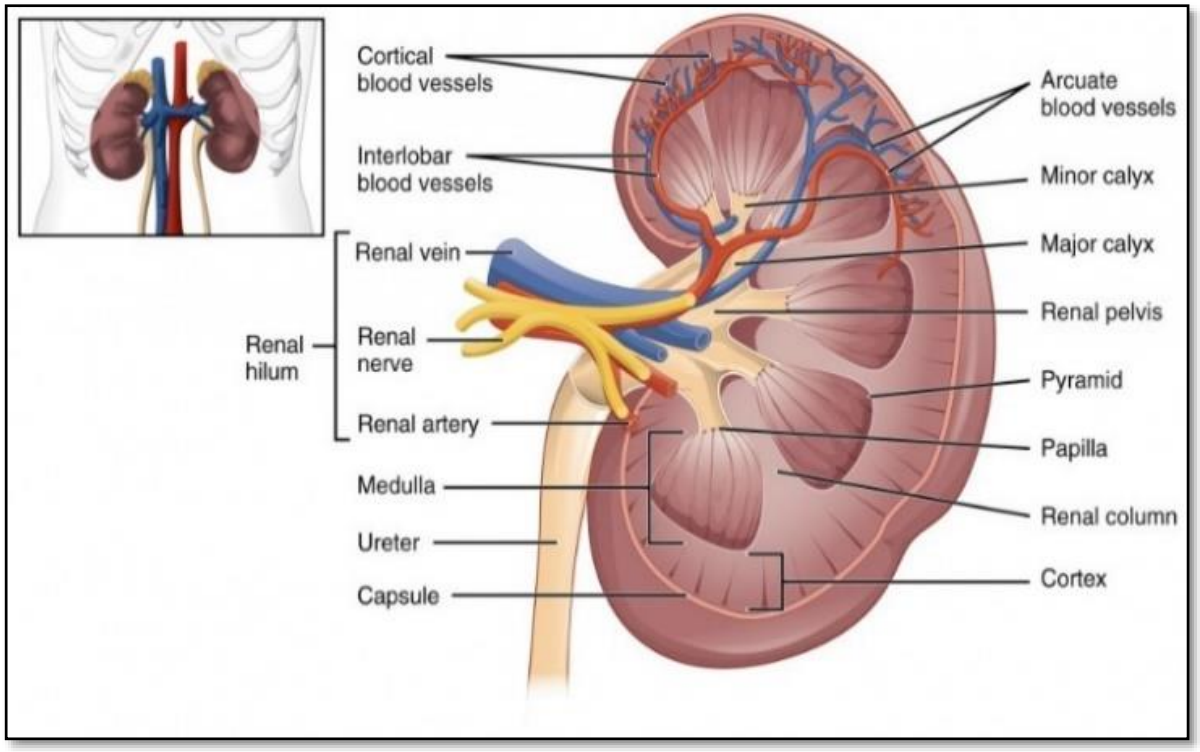

Figure 03: left kidney anatomy [03]

1.1.2. Kidney's microscopic anatomy

On the microscopic level, the nephrons are the functional and structural urine-producing units of the kidney present in the parenchyma (the cortex and medulla) containing about 1 to 1.5 million nephrons per kidney (figure 04). Each nephron consists of 2 parts (Radi, 2019):

- **Renal corpuscle**

Renal corpuscle consists of the glomerulus and Bowman's capsule, which is contained exclusively in the cortex. The glomerulus structure composed of a network of capillary loops, this is supplied by an afferent arteriole which penetrates through the vascular pole of the corpuscle and is drained by an efferent arteriole. Afferent arterioles supply blood to the glomerulus, while efferent arterioles exit the glomerulus. The opposite pole is called urinary due to the lumen of Bowman's capsule which is continuous with that of the proximal convoluted tubule. The corpuscle therefore has the function of filtering the blood that arrives, of developing an ultrafiltrate from it, also called primitive urine.

The glomerular filtration barrier (size, shape, and charge selective) has 3 layers: the fenestrated endothelium, a negatively charged glomerular basement membrane (GBM), and the podocytes. In order to perform this function, we find Bowman's capsule, which has double layers surrounding the glomerulus. (As the blood passes through the glomerulus, about 20% of the plasma can be filtered into this capsule.) Once the primordial urine is developed, it flows to the urinary pole of the body to join the tubular system. (Boccara, 2015; Khan *et al*., 2013; [04]).

- **Renal tubule**

Renal tubule is following the renal corpuscle it is made up of several segments which are lined with epithelial cells that includes the proximal convoluted tubule, the loop of Henle, the distal convoluted tubule, and finally the collecting duct, whose main function is reabsorption and secretion of certain substances. Thus, there is a difference in the structure and function of these cells along the length of the tubule. These phenomena occur with the peritubular capillaries that surround each tubule. The tubular system will transform the primitive urine into definitive urine

by a process of secretion and reabsorption. At the level of the urinary pole, Bowman's capsule leads to the first part of the tubular system which is the proximal convoluted tubule, a tube which at the level of its initial part meanders a lot, this one followed by a more rectilinear part which will lead to a another tubule, the loop of Henlé, a hairpin structure which has a descending branch then an ascending one, leading to the following tubule, the distal tubule, sinuous tubule like the proximal convoluted tubule but shorter and, where part of this tubule comes into contact with the vascular pole of the renal corpuscle and forms the juxtaglomerular apparatus. (Boccara, 2015; Khan *et al*., 2013; [04]).

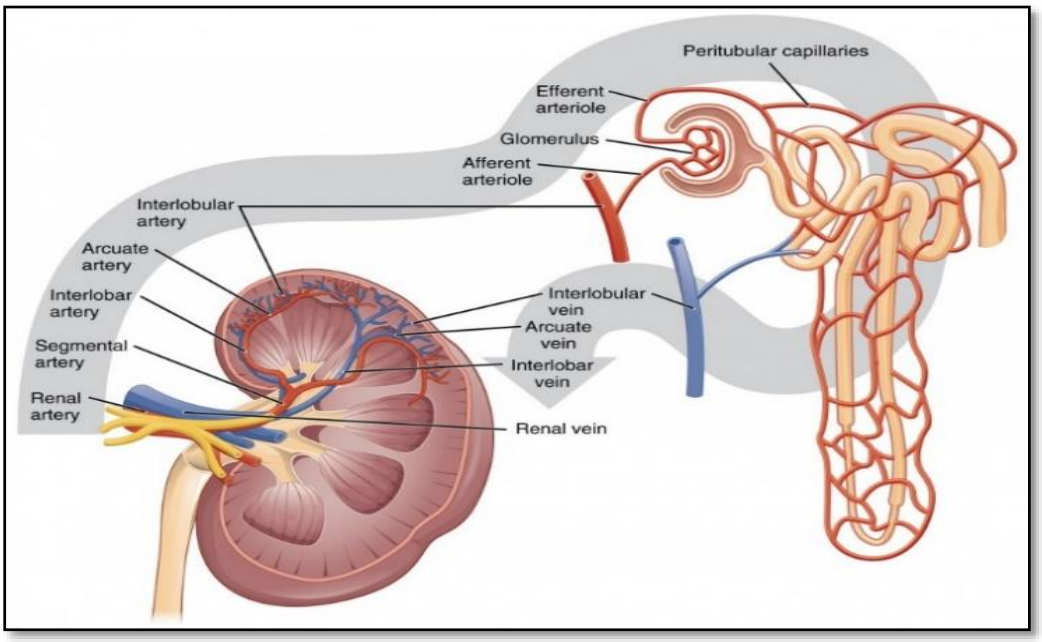

Figure 04: nephrons and blood Flow in the Kidney [05].

1.2. Renal functions within the organism

According to le particulier santé et Zimmer , 2020, the kidney's function has traditionally limited as organs that excreting waste. Although they do indeed excrete waste, a large spectrum of other crucial functions are assured by kidneys such as assuring bone integrity and helping to maintain blood pressure. As they carry out these functions the kidneys work cooperatively and interactively with other organ systems, particularly the cardiovascular system [06]. A blood vessel lines the tubing. As the filtered fluid moves along the tubule, the blood vessel reabsorbs most of the water, as well as the minerals and nutrient the body requires: including sodium and potassium

intake from food, their lack or excess can cause severe complications. The tubule helps likewise eliminating excess acid in the bloodstream. The remaining fluid and waste in the tube turn into the urine, which enable the maintain of the hydric balance as well as neutral blood pH (7,38- 7,42) of the organism (Alcázar Arroyo,2008).

The renin-angiotensin-aldosterone system is a hormonal system located in the kidney plays a primordial role in physiological processes (balance of sodium in the blood, blood volume and blood pressure) and pathological (hypertension, inflammation, fibrosis) Renin, considered as a hormone, is produced and secreted by the granular cells of the juxtaglomerical apparatus. It acts on angiotensinogen (produced by the liver), to form angiotensin I (a decapeptide). This decapeptide, produced by the lungs as a carboxypeptidase, angiotensin-I-converting enzyme I (ACE), will be converted to angiotensin II (an octapeptide). The angiotensin II produced will interact with receptors with 7 transmembrane domains coupled to G proteins, Angiotensin II receptor type 1(AT1R) and AT2R. The ligand-receptor interaction leads to a variety of physiological responses such as vasoconstriction (blood vessels and arterial effects) inducing an increase in blood pressure, the secretion of hormones such as aldosterone and antidiuretic hormone (Veeren,2021; Patel *et al*.,2017).

Kidney is also the organ responsible of the Human erythropoietin (EPO) realizing which is an N-linked glycoprotein consisting of 166 amino-acids (AA) that is produced during the adult life and acts both as a peptide hormone and hematopoietic growth factor (HGF), stimulating bone marrow erythropoiesis. EPO production is activated by hypoxia and is regulated via an oxygen-sensitive feedback loop. EPO acts via its homodimeric erythropoietin receptor (EPO-R) that increases cell survival and drives the terminal erythroid maturation of progenitors (BFU-Es) and Colony Forming Unit E (CFU-Es) to billions of mature RBCs. This pathway involves the activation of multiple erythroid transcription factors, such as GATA1, FOG1, TAL-1, EKLF and BCL11A, and leads to the overexpression of genes encoding enzymes involved in heme biosynthesis and the production of hemoglobin (Tsiftsoglou, 2021).

Additionally, kidneys are also involved in the metabolism of Vitamin D. In this process, vitamin D is released into the skin from 7-dehydrocholesterol. It undergoes a first hydroxylation in the liver to give 25-hydroxy-vitamin D. Then a second hydroxylation takes place in the kidney

to give 1, 25-dihydroxy-vitamin D3: the active and circulating form of vitamin D. Vitamin D actually plays a crucial role in maintaining calcium/phosphate levels within the body (Bikle, 2014).

1.3.Chronic kidney disease

During its lifetime, the kidney is likely to undergo various anomalies on different levels which will absolutely induce alterations in different renal functions, the people affected present what is called chronic kidney disease (CKD). CKD is a progressive disease characterized by long-term silence with no chance of recovery. It is defined as a significant decrease in renal function for more than three months regardless of its cause (biological, morphological or histological anomalies) (Ruiz-Ortega *et al*., 2020). The CKD must be distinguished from chronic renal insufficiency (CRI), in general the CRI: progressive and irreversible decrease of the glomerular filtration flux, results from the evolution of CKD to advanced stages (Corentin, 2022). CDK affects about 10% of the global adult population and more than 30% of people over 70 years of age (Kpemissi *et al*., 2019; Stengel *et al*., 2016).

1.3.1. Classification of chronic kidney disease

A patient's kidney function is assessed according to disease causes (table 01), the level of glomerular filtration rate (GFR) and albuminuria category (table 02), also known as the CGA classification (Ruiz-Ortega *et al*., 2020). GFR corresponds to the volume of fluid filtered by the kidney per unit of time, expressed in mL/min/1.73m2, a GFR less than 60ml/min/1.73m2 persistent for three months or more indicates the presence of a CKD. The GFR estimation is based on the determination of creatinine, where three formulas can be used i.e.: the CKD epidemiology collaboration equation (CKD-EPI), the Cockroft and Gault formula and the modification of diet in renal disease equation MDRD formula (Inker et Tiatan, 2021).

Table (01): Different causes of the CDK (Corentin, 2022).

Cause	Percentage
Vascular nephropathy (hypertension)	25%
Diabetic nephropathy (type 02)	22%
Chronic glomerulonephritis	11%
Hereditary nephropathy (autosomal dominant polycystic kidney disease)	08%
Chronic interstitial nephropathy	05%
Nephropathy of undetermined origin	16%

Table 02: CKD classification according to GFR stages (Crasto *et al*., 2021)

GFR(ml/min/1,73 m²)	**MCR stage**	**Definiton**
>90	1	Normal GFR
60 To 90	2	GFR slightly decreased
30 To 59	3	Moderate chronic renal failure
15 To 29	4	Severe chronic renal failure
< 15	5	End-stage chronic renal failure

1.3.2. Risk factors of chronic kidney disease

Part of the population shows risk factors for CDK (table 03), so an early identification is required for these persons to be screened regularly (Corentin, 2022).

Table (03): risk factors of the CDK (Corentin, 2022)

CDK risk factors
- Diabetes
- Arterial hypertension
- Age > 60 years old
- Obesity (BMI > 30 kg/m2)
- Atheromatous cardiovascular disease
- Heart failure
- Auto-immune disease (lupus…etc.)
- Urological affections (obstructive uropathy …etc)
- Smoking
- Family history of kidney disease that progressed to the Insufficiency stage.
- Terminal Stage Kidney Disease
- Exposure to professional toxins (lead, cadmium, mercury)
- Previous nephrotoxic treatment (in particular NSAIDs)
- antecedent of acute nephropathy

1.3.3. Pathophysiology of chronic kidney disease

Even though several different etiologies may lead to the CKD (table 01), the pathophysiology of progression to end chronic renal insufficiency (ECRI) has a common pathophysiologic process. An understanding of this process makes it possible to better understand the value of proper monitoring of the CKD. CKD has a common histological appearance including glomerulosclerosis, arteriosclerosis and interstitial fibrosis associated with tubular atrophy. Thus, adaptive nephron changes after an initial lesion are assumed to be “poorly adaptive” resulting in scarring. Then, in the absence of capacity to produce new nephrons, the remaining nephrons hypertrophy perpetuating a vicious circle leading to ECRI. The main mechanisms involved are: hemodynamic factors (HTA and glomerular hypertension), renin-angiotensin aldosterone, and

growth factors such as Transforming growth factor beta (TGF-beta), loss of podocytes, proteinuria and dyslipidemia (Ruiz-Ortega *et al*., 2020; Charles et Ferris., 2020).

1.3.4. The consequences of chronic kidney disease within the organism

The kidney as seen above performs a number of functions within the organism. When CKD appears, leads to impairment of the different functions that the kidney performs in the body causing complications, particularly when the kidney cannot adapt to the disease generated. If so, numerous problems will arise. Among these complications, we notice the buildup of many substances inside the body, including toxins and nitrogenous wastes (Dobrek, 2022). Kidney's dysfunction conducts also to a hemolytic disorders, indeed, the CKD leads to a deficiency in the production of (EPO) which will cause a normochromic normocytic aregenerative anemia. Although this anemia is primarily due to the EPO, it is of multifactor origin, other factors are involved such as: uremic toxins leading to a decrease in erythropoise, possible martial deficiency and vitamin deficiency (Batchelor *et al.*, 2020; Mikhail *et al*., 2017).

A metabolic acidosis that corresponds to a primary reduction in serum bicarbonate (HCO^{-}_{3} concentration, a secondary decrease in the arterial partial pressure of carbon dioxide ($PaCO^{2}$) of approximately 1 mmHg for every 1 mmol/l fall in serum (HCO^{-}_{3}) concentration, and a reduction in blood pH in the blood reducing the plasma PH due to H+ accumulation, is also can be caused by CDK (Kraut et Madias, 2010).

It is known that kidneys are the major organs that play a predominant role in the control of the fluid and electrolyte balance through the preserving of the equilibrium of hydric and sodium balance, parallel to maintaining of the osmolality as well as volemia, it therefore contributes to the hydromineral homeostasis of the medium, a renal abnormality leads by consequence to a hydro-electrolyte disturbance (Boccara,2015).

On the bone level, rickets is a metabolic bone disease, which refers to inadequate mineralization of growing bones caused by abnormal metabolism of calcium, phosphorus, and/or vitamin D occurs when there is inadequate phosphorus intake at intestine or excessive renal wasting (Sun *et al*.,2020).

A correlation between renal dysfunction and the onset of cardiovascular events has been well proven, especially with regard to the accumulation of homocysteine (Hcy): a sulfhydryl-containing amino acid, which is not acquired through the diet, but rather synthesized as an intermediate

metabolite in the methionine cycle. Hcy is present in plasma, with normal levels between 5 and 15 µmol/L, a slightly elevated level between 15 to 30 µmol/L, moderate from 30 to 100 µmol/L and a value > 100 µmol/L classified as severe hyperhomocysteinemia (HHcy). HHcy has been associated with inflammation and atherosclerosis and is considered an independent risk factor for CVD. The accumulation of this acid is mainly due to abnormal metabolism that takes place in the kidneys (Guieu *et al.*, 2022; Boccara, 2015).

Several studies showed that renal dysfunction changes the level, composition and quality of blood lipids in favour of a more atherogenic profile we refer to a dyslipidemia. Patients with advanced CKD have a characteristic lipid pattern of hypertriglyceridaemia and low high-density lipoprotein (HDL) cholesterol levels but normal-low-density lipoprotein (LDL) cholesterol levels. In the general population, a clear relationship exists between LDL cholesterol and major atherosclerotic events. However, in patients with end-stage renal disease, LDL cholesterol shows a negative association with these outcomes at below average LDL cholesterol levels and a flat or weakly positive association with mortality at higher LDL cholesterol levels (Ferro *et al*., 2018).

1.3.5. Genetic side of CKD: role of miARNs

A recent data indicate the involvement of microRNAs (miRNAs) in the development of renal diseases pathophysiology, knowing that miARNs are an abundant class of small uncoding RNA which play an essential role in post-transcriptional gene expression's regulation by binding to the messenger RNA 3'-UTRs sequence. This combination leads to the inhibition of the targeted RNAm's translation and consequently the inhibition of the target gene expression which regulate various physiological and pathological processes, including cell differentiation, proliferation, apoptosis, and metabolism (Liu *et al.*,2022; Amrouch *et al*.,2011).

The biogenesis of miRNAs is complex. RNA polymerase II transcribes the gene encoding a miRNA in the nucleus to produce primary miRNA (pri-miRNAs), which is then cleaved into double-stranded miRNA precursor (pre-miRNAs) by RNase III enzyme Drosha and its partner DGCR8. The pre-miRNA is then exported into the cytoplasm by the Ran-GTP and Exportin-5, where pre-miRNA is further cleaved into mature miRNAs that contain 21–25 base pairs. miRNAs regulate gene expression by binding to target mRNAs and then recruiting the RNA-

induced silencing complex (RISC) to induce miRNA degradation or prevent miRNA-protein translation(figure 05) (Liu *et al*.,2019).

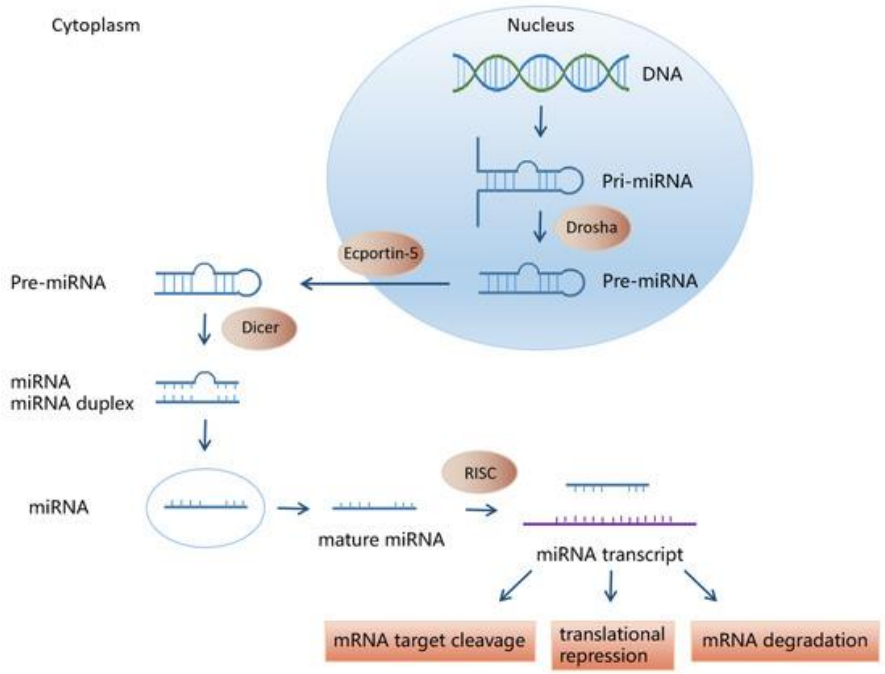

Figure 05: miRNA biogenesis process (Jinmeng *et al*.,2022)

The miRNAs intervene in the MRC are multiple and are organized in different families such as the miARN-let 7, miR-31, miR -126, etc each familly of them is characterized by a level and mechanism of action that is appropriate for them (Peters *et al*.,2020).

miRNA-21 is one of the most miRNAs characterized miRNAs at present, it is involved in different physiological and pathological situations, beside the fact that it is quantitatively and fonctionnaly well determined with a genetic locus identified (Figure06) compared to the other types of miRNA still not well known. Actually, several studies showed that miRNA-21 is implicated in the development of a large number of both acute and chronic renal diseases, also there has been a lot of research evidence for the role of miRNA-21 in diabetic nephropathy development through its excessive effect on different signaling pathways such us phosphatase and tensin homolog deletions in chromosome (PTEN), Mitogen-activated protein kinase 3 (MAPK3)…etc (Figure 07)(Larrue et *al.*,2022)

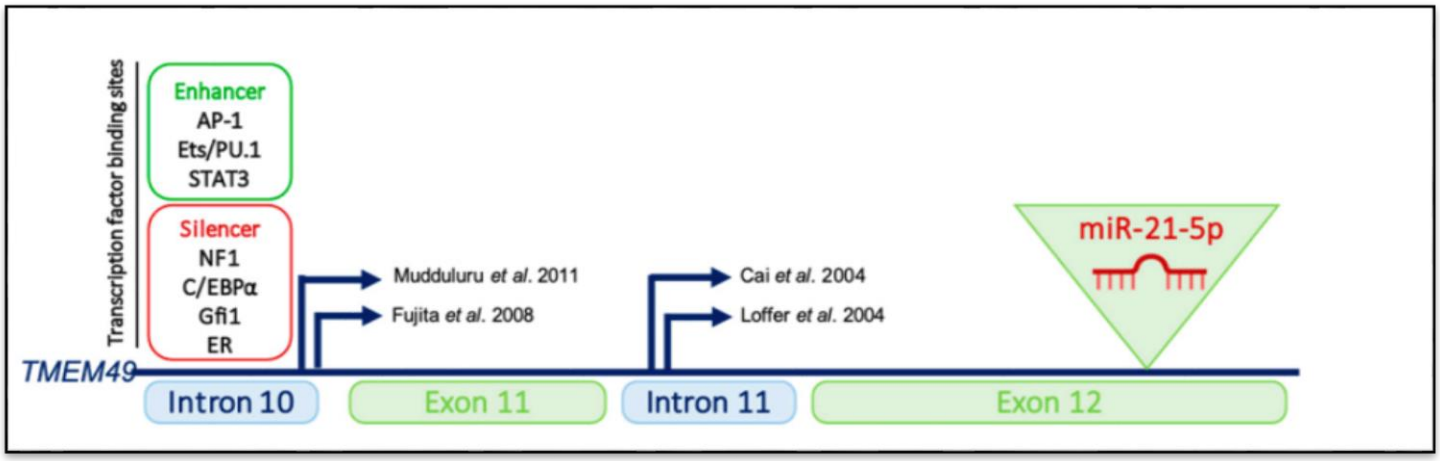

Figure 06: Genetic locus of pri-MiR-21 on chromosome 17q23.2 (Larrue et *al.*,2022)

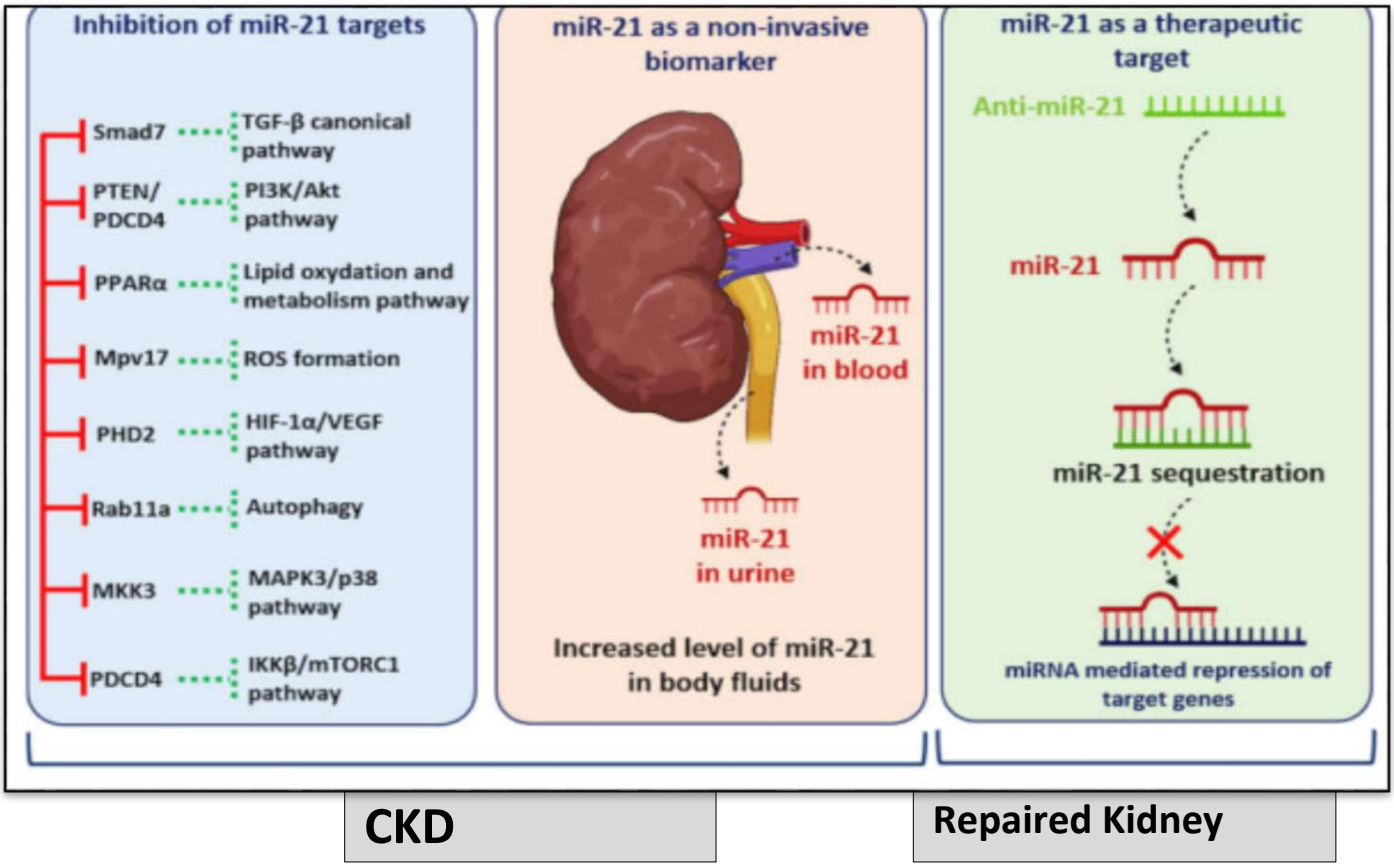

Figure 07: miR-21 role in CKD (Larrue et *al.*,2022).

1.3.6. Management of chronic kidney disease

It is required to mention that from stage 4 of CDK, patients have to be informed of the various possible replacement techniques: peritoneal dialysis, hemodialysis, or transplantation of a kidney from a deceased or living donor (Enjalbrt-Auneau, 2020).

1.3.6.1. CKD diagnosis and treatment approaches

There are still major challenges regarding the early diagnosis and treatment of chronic kidney disease (CKD), which is in part due to the fact that its pathophysiology is very complex and not clarified in detail. In general, the GFR measurement is based on the determination of serum creatinine that used in the GFR calculation equations, Cockroft and Gault or MDRD. Whereas the fact that creatinine alone is not such an exact parameter in the evaluation of GFR because its value in the blood depends on various factors other than renal ones such as age, sex, muscle mass, diet, race...etc. That's what incites scientists to develop a new equation based on a new sensitive parameter to detect the CKD diseases which depends only on renal factors, this parameter is the cystatin C (CysC). CysC is a is a non-glycosylated, 13.3-kDa protein belonging to cysteine protease inhibitors, newly discovered endogenous biomarker of kidney damage which can be used to detect early as well as acute and chronic renal failure unlike creatinine which only enable the detection of CKD in late stages, this is due to its filtration at the glomerular level then the complete catabolization in the proximal tubules, on the contrary creatinine it is not secreted. Its urinary excretion is thus very low. Studies showed that the determination of cysC in serum and urine can be routinely used for diagnosis and treatment (Meeusen *et al*., 2022; Inker *et al*., 2021; Tokarzewicz et Gorodkiewicz, 2015).

A study realized by Meeusen and his team on 2022 showed that the most recent equation uses the two parameters: creatinine and CysC together is the most sensitive and accurate for the estimation of the GFR: CKD: EPI equation (Figure 08) (Meeusen *et al*., 2022).

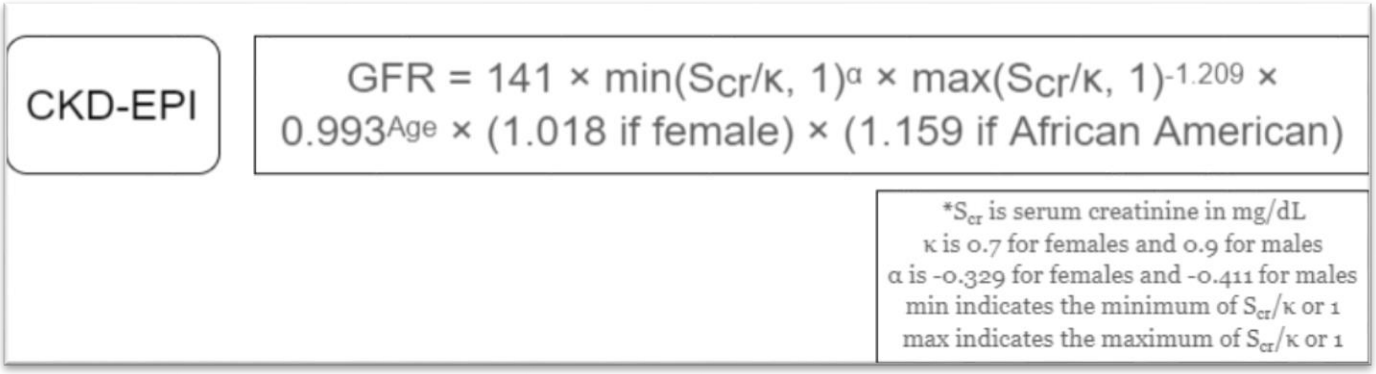

Figure 08: CKD-EPI equation [07]

Given that aberrant miRNA expression has been causatively linked to a vast array of CKD, the development of miRNA-based therapies has become a major goal of the future research, of these miRNAs (Larrue *et al.,* 2022). miR-21 has received particular attention given its abundant baseline in particular, the successful pharmacological silencing of miR-21 has been achieved in various experimental models of acute kidney injury (AKI), as well as in chronic glomerular and tubulointerstitial diseases. This includes in particular common kidney disorders such as diabetic nephropathy or renal fibrosis (Zhao *et al*.,2021).

The Manipulation of the specific miARNs's activity in the kidney was performed by administering inhibitors to block the function of miARN or mime it to restore miARNs levels. Many miRNAs are activated by stress (acute renal injury for example) and appear to be inactive in healthy cells, which is a therapeutic advantage in developing safe treatments that target miRNAs such us anti-miRNAs oligonucleotides capable of penetrating cells after systemic administration to suppress translation. Similar efforts have been made to generate copies of miRNA. Although microRNAs are generally resistant with a long half- Several advances have been made to improve the stability of in vivo RNA by molecular modification of the skeleton. In addition to anti-miR and mimics, inhibition of miRNAs can be achieved by expressing target miRNA sequences capable of capturing pathogenic microRNAs (miRNA sponges) or hairpin RNA plasmids that suppress the expression of miRNAs by RNA interference. Effective suppression can also be achieved by using complementary oligonucleotides of the 3'-UTR end of target mRNAs or the sequence of miRNAs themselves (Larrue *et al.,* 2022; Favroult, 2019).

However, several limitations, such as safety issues and administration methods, must be overcome before miRNA-based treatments for chronic kidney disease can be translated into clinical practice. Ideally, the target miRNA should be kidney-specific to avoid adverse effects on other tissues and organs. To minimize side effects, treatment should only affect a cell target (Larrue *et al.*, 2022; Favroult, 2019).

2. Diabetes mellitus

As mentioned earlier, diabetes is one of the most important causes of CKD. Diabetes mellitus (DM) is actually refers to a collection of metabolic disorders characterized by chronic hyperglycaemia. The condition arises from either the inability of the body to produce enough insulin or the inability of insulin to function effectively, or in many cases, both (Petersmann *et al.*, 2018).

2.1. Diabetes classification

It can be categorized into general groups, and the first category is Type 1 diabetes (T1D). This type is caused by the destruction of β-cells by an autoimmune response, which often leads to a complete lack of insulin. It also includes latent autoimmune diabetes of adulthood, a form of T1D that is diagnosed later in life. Type 2 diabetes (T2D) occurs when there is a gradual decline in the β-cells' ability to secrete insulin, often in the context of insulin resistance. Diabetes is usually diagnosed in adults and can often be managed through lifestyle modifications, such as changes in diet and exercise, along with medications as needed. A various types of diabetes may result from genetic mutations or damage to the pancreas due to medication or disease. One example is monogenic diabetes, which is caused by a single gene mutation that affects insulin production. Another type is gestational diabetes, which is a temporary form of diabetes that may develop during pregnancy (American Diabetes Association, 2021).

2.2.Diabetic kidney disease

Diabetic kidney disease (DKD) is typically diagnosed based on clinical assessment. This involves confirming the presence of diabetes as the first step, followed by demonstrating the presence of kidney disease, which can be defined by either albuminuria or decreased GFR.

While albuminuria has traditionally been defined using 24-hour urine albumin excretion rate (UAER), the most common method for testing albuminuria is now through a random spot urine albumin-to-creatinine ratio (UACR). The use of 24-hour or timed urine samples in clinical practice can be cumbersome for patients and may introduce inaccuracies due to incomplete collection. (Microvascular Complications and Foot Care, 2020).

2.2.1. Pathophysiology of diabetic kidney disease

DKD is a multifactorial condition characterized by various structural, physiological, hemodynamic and inflammatory processes that lead to a decline in the GFR. The Sodium-glucose cotransporter-2 inhibitors (SGLT2) transporter plays a central role in the initiation of many of these pathophysiological abnormalities by increasing glucose and sodium reabsorption in the proximal tubule.

However, inhibiting the SGLT2 transporter can reverse these disturbances and significantly slow the progression of DKD (DeFronzo *et al*., 2021).

Multiple physiological disruptions suggests that once DKD is established, multiple therapeutic agents may be needed to address the underlying abnormalities. Hyperglycemia is the main factor underlying the development of DKD. Patients with normal HbA1c levels do not develop DKD, but microalbuminuria can occur in individuals with "prediabetes". The proportion of individuals with microalbuminuria who progress to macro-albuminuria and eventually to CKD while HbA1c remains in the prediabetic range is unknown. Maintaining HbA1c levels below 6.5% is recommended to prevent the development of DKD, and intensive glycemic control can reduce the incidence of albuminuria by 50% and the risk of microvascular complications by 37%. However, the level of albuminuria and GFR at which intensive glycemic control fails to slow the

progression of established DKD is unclear. Other methods, such as kidney biopsy, may be needed to identify patients at risk of DKD development and progression (Amorim *et al*.,2019; Lytvyn *et al*., 2019).

The development of DKD is significantly influenced by hypertension, following hyperglycemia. (Xie *et al*., 2016). Patients with advanced DKD who have normal blood pressure show a slower progression of the disease than those who are hypertensive. However, in a study that aimed to control cardiovascular risk in patients with T2DM, achieving a systolic blood pressure target of less than 120 mmHg did not reduce cardiovascular events. Moreover, this approach was linked with a higher incidence of hyperkalemia and increased levels of serum creatinine (Lytvyn *et al*., 2019).

2.2.2. Treatment of diabetic kidney disease

Treating high blood sugar in patients with T2D and impaired kidney function is challenging for several reasons, which may result in avoiding or adjusting the dosage of certain anti-diabetic drugs (Fried *et al*., 2021).

First, the liver and kidneys are major sites for drug metabolism and elimination. This means that levels of drugs that are broken down or excreted through the kidneys may increase in these patients, leading to a higher risk of adverse effects, such as low blood sugar (hypoglycemia) (Lea-Henry *et al*.,2018).

Second, impaired kidney function itself increases the risk of hypoglycemia, even in non-diabetic individuals, as the kidneys contribute to the production of glucose in the body. Additionally, in individuals with impaired kidney function, hypoglycemia is favored by the presence of acidosis, which limits the liver's ability to compensate for reduced glucose production by the kidneys, as well as by malnutrition and muscle wasting, which decrease the liver's glycogen stores and availability of substances for glucose production (Pugliese *et al*., 2020).

Third, patients with impaired kidney function are often excluded from clinical trials, resulting in limited evidence on the effectiveness and safety of various anti-diabetic drugs in this population, particularly those with very low estimated GFR levels (Pugliese *et al*., 2020).

Finally, compared to patients without kidney disease, those with kidney disease are usually older, have longer duration of diabetes, and often have other comorbidities, such as cardiovascular diseases, and are on multiple medications that may interact with anti-diabetic drugs (Pugliese *et al*., 2020).

The mainstay current treatments of DKD are: renin-angiotensin system (RAS-inhibitor), Mineralocorticoid receptor antagonist (MRA), multidisciplinary treatments and SGLT2 inhibitor.

The RAS inhibitor is the oldest and most prominent medication for the treatment of DKD. In fact, clinical trials have demonstrated the effectiveness of RAS inhibitors in managing DKD. Research has indicated that an angiotensin-converting-enzyme inhibitor (ACE I), can hinder the progression of nephropathy in patients with overt nephropathy in T2DM. Furthermore, large randomized controlled trials (RCTs) have shown the effectiveness of angiotensin II receptor blockers (ARBs) in managing diabetic patients with manifest nephropathy (Koszegi *et al*., 2019).

Both extensive clinical trials and fundamental experiments have provided substantial evidence supporting the effectiveness of RAS inhibitors, specifically ACE-I and ARB, in the treatment of DKD. However, they are not completely effective in preventing the development of end-stage kidney disease (ESKD) and may even increase the risk of complications. As a result, there was anticipation for the development of new drugs to address DKD (Yamazaki *et al*.,2020).

Consistent findings from clinical trials indicate that the use of SGLT2 inhibitors can provide renal protection by reducing the rate of decline in estimated GFR and delaying the onset or progression of albuminuria. While sustained improvements in glucose homeostasis can reduce the risks and severity of renal complications in T2D regardless of the glucose-lowering medication used, the renal benefits associated with SGLT2 inhibitors appear to be more pronounced and faster in onset, and are not solely dependent on their glucose-lowering effects (Bailey *et al*., 2022). SGLT2 inhibitors decrease both fasting and postprandial plasma glucose concentrations, resulting in improved β-cell function and enhanced insulin sensitivity. They work by reducing the renal threshold for glucose excretion and increasing glucose uptake by kidney cells. SGLT2 inhibitor

can reverse the harmful metabolic effects caused by high glucose levels by reducing the amount of glucose in the blood (Warren *et al*., 2019).

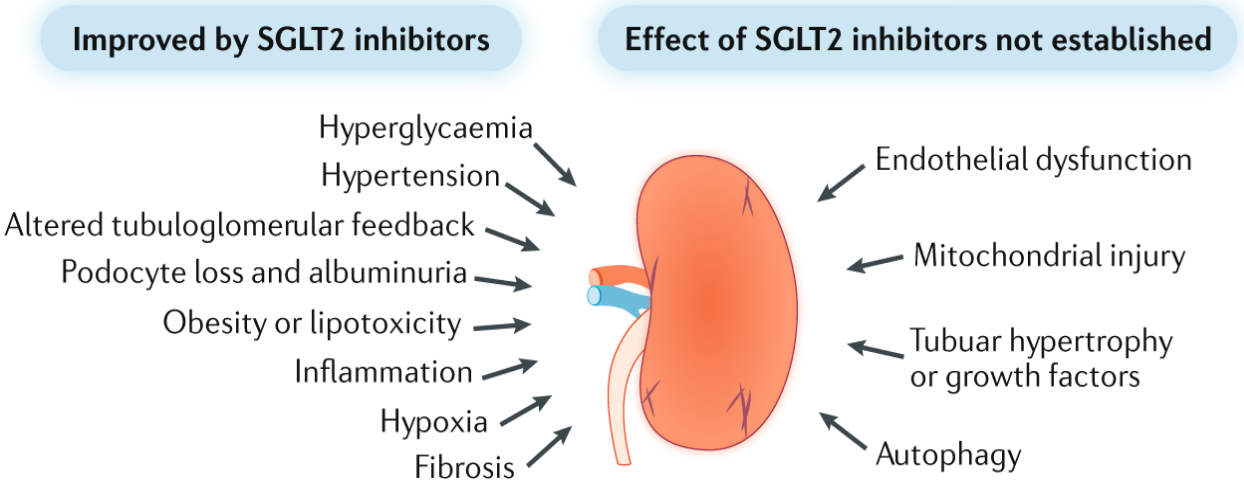

Figure09: Effect of SGLT2 inhibitors (Warren *et al*., 2019).

3. Screening of CKD and prediabetes

According to the United States (US) commission on chronic illness 1968, screening is define as the presumptive identification of unrecognized disease or defect by the application of tests, examination or other procedures, which can be applied rapidly. Screening test sort out apparently well persons who apparently have a disease from those who probably do not (Holland et Stewart, 2005).

When examining a screening program, several factors should be considered: the natural history of the disease of interest, screening test characteristics, and how the screening test and available treatments influence the natural history of disease. When developing a screening trial protocol, critical elements include the identification of the target population, determination of the screening regimens to be compared, and selection of outcomes to be measured and appropriate corresponding study designs (Lee et al,. 2016).

Screening for (CKD) involves the identification of individuals who may be at risk or already have early stages of kidney damage. It aims to detect CKD early, allowing for timely interventions to slow down the progression and prevent complications. Screening typically includes laboratory tests such as GFR and Urine albumin-to-creatinine ratio **(**UACR). A GFR below 60 mL/min/1.73m² for three months or longer indicates reduced kidney function and potential CKD. These tests help assess kidney function and detect signs of kidney damage. Early detection through screening enables appropriate management strategies to be implemented, potentially improving outcomes and quality of life for individuals with CKD. Remember, early detection and timely management play a crucial role in preventing the progression of CKD and reducing the risk of complications. Regular screenings and open communication with healthcare professionals are essential for maintaining kidney health. (Grill et Brimble.,2018).

In DKD, an effective screening is crucial due to the high morbidity and mortality rates associated with this affection. Patients with T2DM and those with T1DM for 5years or more should undergo an annual assessment of GFR and albuminuria using the UACR. However, despite the importance of screening, less than 50% of individuals with diabetes receive testing for abnormal urine albumin levels. It is recommended to examine albuminuria, and if elevated, confirm with a separate measurement within 6 months (Selby et Taal, 2020).

In this paper, we target to detect the prevalence of CKD as well as prediabetes among normally healthy young subjects (18-35 years old). Moreover, we strive to raise awareness among them about the distinctiveness and severity of these diseases, highlighting the need for early recognition and intervention.

For this aim, our study is subdivided into three major parts:

- The first is the Introduction includes the theoretical part of the theme
- The second describes the materials and methods used during the study.
- The third part is about the result's presentation besides their discussion
- We finish with a general conclusion and perspectives followed by the references list than the list of the attachments.

Materials and methods

1. Study goals

Since the CKD is a silent serious disease with irreversible consequences and high public health stakes, that prediabetes is one of its most important outcomes, an early diagnosis makes it possible to reduce the evolution of this disease towards CRI, it is for this reason a screening is indicated. Our present study aims to detect the prevalence of CKD as well as prediabetes among normally healthy young subjects (18-35 years old) and to sensitize them to the particularity and severity of these diseases.

2. Study protocol

2.1. Type/ location/duration of the study

The present study is a prospective experimental study that consists in screening that have covered 100 young subjects (18-35 years old) who live in the city of Guelma of which the majority of the cases are students of the 8 Mai 1945 university of Guelma. It included a survey (attachment 1) containing questions that the participants required to answer as well as a series of biochemical analyzes that will be determined afterwards. The blood sample and the biological analyzes of the biochemical parameters concerned were carried out in the private laboratory Dr Nouar. The realization period of this study is approximately 3 months (from March to June).

2.2.Recruitment of the subjects participating in the screening

The recruitment of the target subjects was performed following an announcement in different ways (direct contact, social networks,.. etc.).

2.3.Patient inclusion and exclusion criteria

The criteria concerning the subject's inclusion who could participate in this study were: being between 18 and 35 years of age, non-diabetic diagnosed and with no known kidney disease. While people over 35 and pregnant women were excluded from the study.

2.4.Data collection

The data collection was carried out at the laboratory Dr. Nouar during the blood sample, by answering the survey questions in order to collect the necessary information

The main information collected was about:

- Personal information: name, age, study level, occupation, family situation.
- Anthropometric parameters: weight, height, size, body mass index (BMI), systolic and diastolic arterial pressure
- Smoking state.
- Physical activities.
- Alimentary habits.
- Personal medical history.
- Family medical history.
- Medication use: subsequent or ongoing

2.5.Collection of blood and urine samples

The blood sample was collected for each patient from blood venous under sterile conditions. It is collected in heparin tubes containing lithium heparin anticoagulant (figure10,11). The urine sample was collected in dry tubes (figure 11).

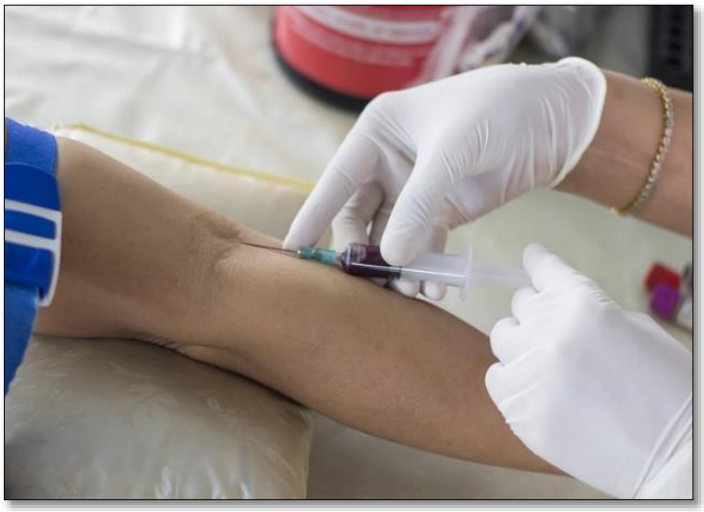

Figure 10: venous blood sample

Figure 11: Heparine (green) and dry tubes blood sample

3. Methods

3.1.Fasting blood glucose

Glycemic assay is a biochemical parameter based on the measurement of plasma glucose concentration. Which is carried out mainly from the veins (blood test), capillaries (glycemia reader and test strips) or interstitials. Normal blood glucose is between 0.70g/l and 1.10g/l fasting. Hypoglycemia < 0.60g/l and hyperglycemia > 1.10g/l (Tchuem Tchuenté, 2021).

3.1.1. Preparation of the sample

- fasting at least 12h before blood sample
- A blood sample from the vein on the heparin tube
- Centrifugation of the tubes 30000 turns for 3 min by a centrifuge (attachment 2).

3.1.2. Glucose-TR (Spinreact)

- Glucose determination using Spinreact (figure 12)

Reagents

Table 4: Glucose dosage reagents (attachment 2)

R1 **Buffer**	TRIS PH 7.4 Phenol	92mmol/L 0.3mmol/L
R2 **Enzymes**	Glucose oxidase (GOD) Peroxidase (POD) 4-Aminophenazone(4-AP)	15000U/L 1000U/L 2.6mmol/L
Glucose CAL	Glucose aqueous primary standard	100mg/Dl

Preparation

- **Working reagent (WR):** dissolution of R2 (one viral enzyme _ R 1(one bottle buffer).
- Cap and mix softly until total dissolution.
- **Stability**: one month after reconstitution in the refrigerator (2-8C°) or 7 days at room temperature (15-25C°) (attachment 2).

Figure 12: Spinreact RT glucose

3.1.3. Protocol

- Preparation of the three tubes:
  - **Blank:** 1 ml WR.
  - **Standard:** 1ml WR + 10 ul standard
  - **Sample:** 1ml WR + 10ul plasma.
- Incubation 10 min at 37c°
- Reading of results using a spectrophotometer (attachment 2).

3.2.Blood creatinine dosage

Creatinine is a waste of the body that comes from the breakdown of muscle creatinine. It is eliminated in the urine by filtration within the kidneys. When the kidneys' ability to dispose of

waste decreases, the amount of creatinine in the blood increases. Its dosage in the blood therefore depends on kidney function but also on muscle mass. Normal values of creatinine are between 06 and 11 for women and 07 and 13 for men (Delanaye *et al*., 2017).

3.2.1. Preparation of the sample

-A blood sample from the vein on the heparin tube

- Centrifugation of the tubes 30000 turns for 3 min by a centrifuge.

3.2.2. Creatinine-J (Spinreact)

Reagents

Table 5: Creatinine dosage reagents (attachment 3).

R1:Picric Reagent	Picric acid	17.5mmol/L
R2: Alkaline Reagent	Sodium hydroxide	0.29mmol/L
Creatinine CAL	Crea aqueous primary standard	2mg/dL

Preparation

- **WR:** mix of an equal quantity of R1 with R2**.**
- **Stability:** one month after reconstitution in the refrigerator (2-8C°) or 7 days at room temperature (15-25C°) (attachment 3).

3.2.3. Protocol

- 250 ml WR1 + 250 ml WR2 + 50ul plasma
- Reading of results using a spectrophotometer (attachment 3).

Figure 13: Spinreact creatinine reagents.

3.2.4. Measurement of creatinine clearance

As the dosage of creatinine is not such a reliable parameter in order to detect CDK, it is usually followed by a measurement of creatinine clearance to confirm the results obtained on the one hand and to determine GFR on the other hand .to calculate the creatinine clearance several methods can be used: Cockroft and Gault equation, MDRD equation (Padgett *et al.*, 2017; Bouccara, 2015).

According to Bouccara, 2015 Clearance creatinine equations are:

- **Crockroft-Gault equation:**

Crockroft-Gault equation = (140-age (years) X weight (kg)/ (creat (mg/dL)X 72)

For women, multiply by 0.85

- **MDRD equation:**

Clearance creatinine MDRD (men) = $186\ (\text{Crea X } 0.885)^{-1.154}\ \text{X age}^{-0.203}\ \text{X } 0.742$

Clearance creatinine MDRD (women) = $186\ (\text{Crea X } 0.885)^{-1.154}\ \text{X age}^{-0.203}$

Clearance creatinine MDRD (black sub) = $186\ (\text{Crea X } 0.885)^{-1.154}\ \text{X age}^{-0.203}\ \text{X } 1.212$

3.3.Urea-Blood dosage

Urea is a waste product that is formed in the liver as a result of protein metabolism. It is primarily excreted by the kidneys through urine. Urea levels in the blood can provide valuable information about kidney function and overall health. Elevated levels of urea in the blood, known as "blood urea nitrogen" (BUN) or "urea nitrogen," can indicate impaired kidney function or other medical conditions. Normal Urea-B is between (0, 15 g/L and 0, 25 g/L)(Landry *et al.*,2020).

3.3.1. Preparation of blood samples

- It considered as an emergency test so the blood samples can be collected after an hour from eating (Fasting is not necessary)

- From the vein into vacutainer tubes with sodium heparin (BD Vacutainer)

- Centrifugation of the tubes 30000 turns for 3 min by a centrifuge (attachment 4).

3.3.2. Urea-B Berthelot (Spinreact)

Reagents

Table 6: Urea-B dosage reagents (attachment 4).

R1	Phosphate PH 6,7	50mmol/L
Buffer	EDTA	2mmol/L
	Sodium salicylate	400mmol/L
	Sodium nitroprusside	10mmol/L
R2	Sodium hypochlorite(NaClO)	140 mmol/L
NaClO	Sodium hydroxide	150mmol/L
R3	Urease	30000U/L
Enzymes		
UREA CAL	Urea aqueous primary standard	50mg/dL

Preparation

- **Working reagent (WR):** Dissolve (→) one tablet R 3 Enzymes in one bottle of R 1 Buffer. Cap and mix gently to dissolve contents.

- **Stability**: 4 weeks in the refrigerator (2-8°C) or 7 days at room temperature (15-25°C)

- **R2 (NACIO)** is ready to use (attachment 4).

-

3.3.3. Protocol

- Preparation of the three tubes:
  - **Blank:** 1 ml WR.
  - **Standard:** 1ml WR + 10 ul standard
  - **Sample:** 1ml WR + 10ul plasma.
- Mix and Incubation 5 min at 37c° or 10 min in room (15-25 c°)

- Add the R2 (NACIO) for each tube:
  - Blank: 1 ml R2
  - Standard: 1ml R2
  - Sample: 1ml R

- For the 2nd time mix and Incubation 5 min at 37c° or 10 min in room (15-25 c°)

- Read The Absorbance (A) of the Sample and Calibrator, Against The Blank. The color Is Stable for at Least 30 Min at (15-25c °)

- Reading of results using a spectrophotometer (attachment 4).

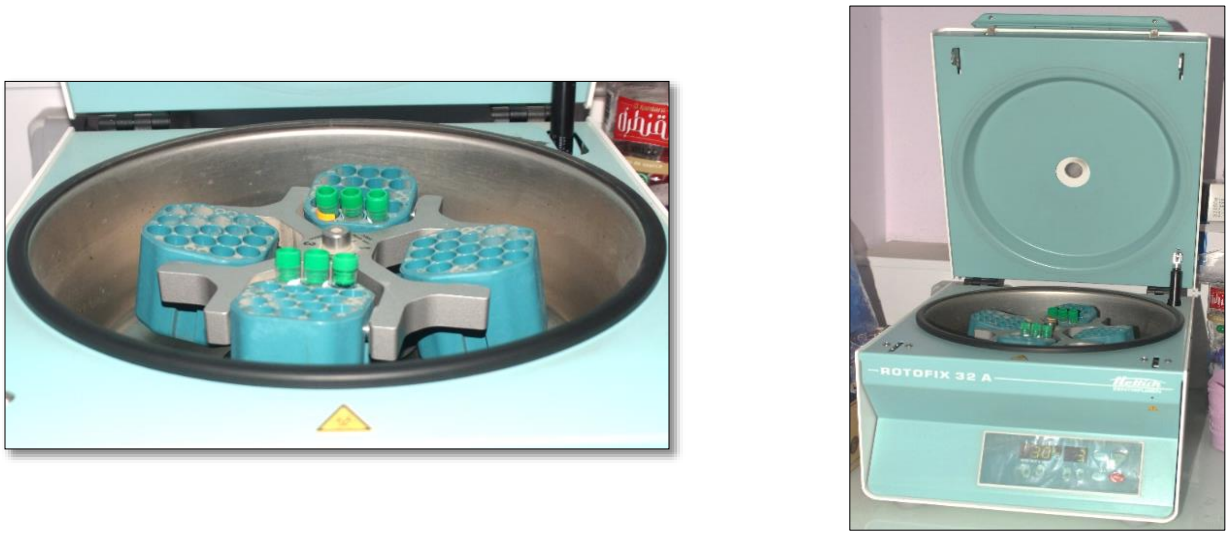

Figure 14: Blood centrifugation to obtain serum

Figure 15: Blank, standard and sample tubes preparation

Figure 16: Tubes incubation at 37C°

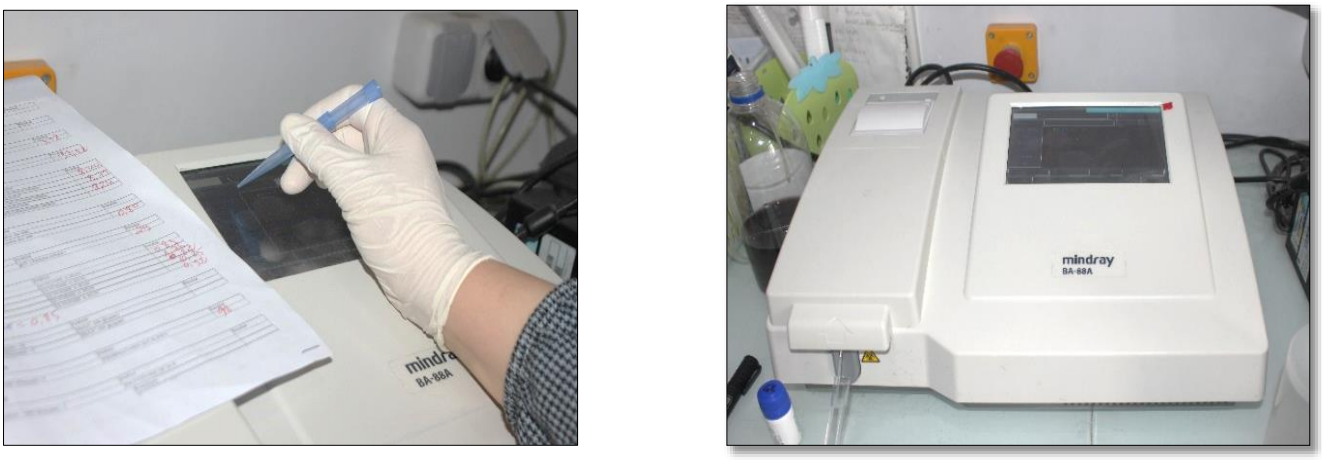

Figure 17: Reading of results using a Mindray spectrophotometer

3.4.Urine chemistry

Urine chemistry is a group of one or more tests done to check the chemical content of a urine sample. The analysis of urine chemistry by test strips is one of the most common tests in a medical office. It allows for the detection of metabolic, hepatic, and renal disorders, as well as urogenital infections (Lanri et Bazari, 2021; [08]).

3.4.1. Preparation of the sample

- Urine Sample

- Patient name identification

- Genital hygiene

- Collect in a dry tube (no detergent residue)

- No centrifugation

- After urination, process the urine as soon as possible (within 2 hours)

- If stored in the refrigerator, allow it to reach room temperature (~30 minutes) before testing.

- Test Strips

- Never reuse or cut the test strips.

- Do not use expired test strips (the expiration date is indicated on the packaging).

- Packaging of Test Strips

- Store in a dry place (< 30°C) and in the original packaging (storage temperature, see packaging).

- Immediately after use, close with the cap to protect from moisture and light

(Lanri et Bazari, 2021; [08]).

3.4.2. Urinary Reagent Strip

The test consists of a strip with reactive zones of dry chemistry, used to qualitatively and/or semi-quantitatively detect various parameters in urine, such as leukocytes, nitrites, pH, proteins, glucose, ketones, urobilinogen, bilirubin, erythrocytes (or blood), and specific gravity (density) (Lanri et Bazari, 2021; [08]).

3.4.3. Protocol

-Wear gloves.

- Timer for calculating the time.

-The test strips are dipped in the urine.

Read visually according to the color comparison chart printed on the side of the container at prescribed time intervals. (1-2min).

-After 1 min, read the results for nitrites, pH, proteins, glucose, ketones, urobilinogen, bilirubin, blood and density.

-After 2 min, read the result for leukocytes.(Lanri et Bazari, 2021; [08]).

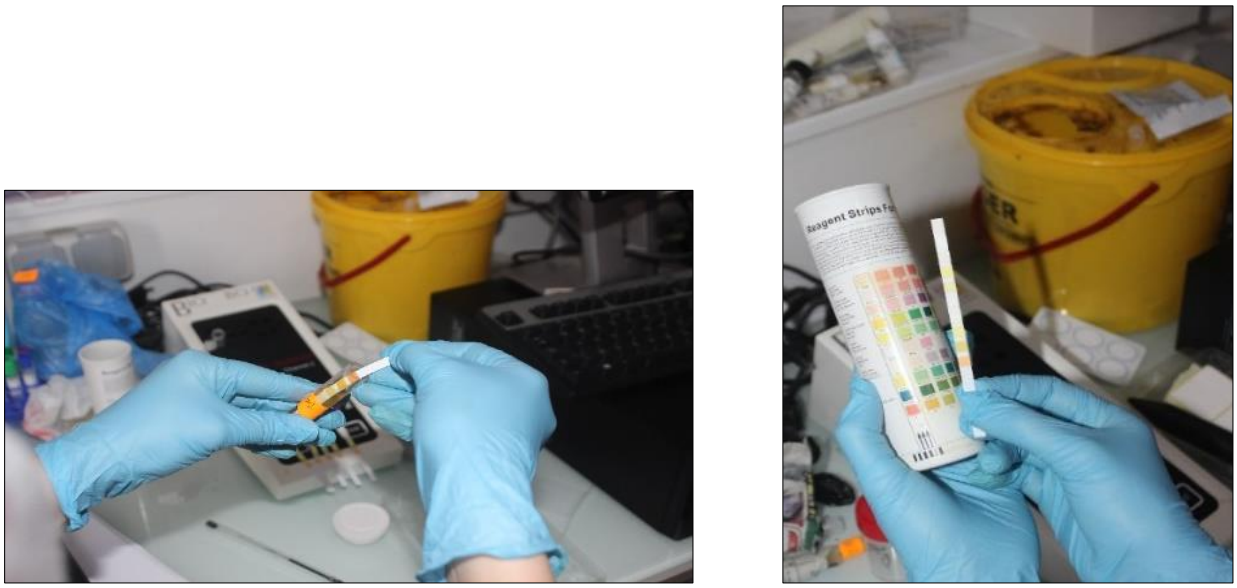

Figure 18: Urine chemistry identification method

Results and discussion

1. The distribution of the results obtained according to the survey' answers

1.1.Distribution according to epidemiological characteristics

1.1.1. According to age and gender

In the present investigation, 100 persons between women (83%) and men (17%) (Figure 19), their age vary between 18 and 35 years (Figure 20) were studied. There is an increased number of subjects that belong to [18-24] because the study was already conducted among young persons and we note also that the gender ratio is about 83/17.

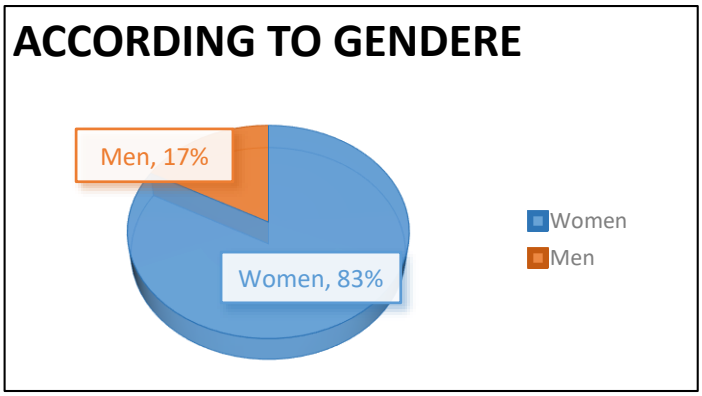

Figure 19: Subject's distribution according to the gender

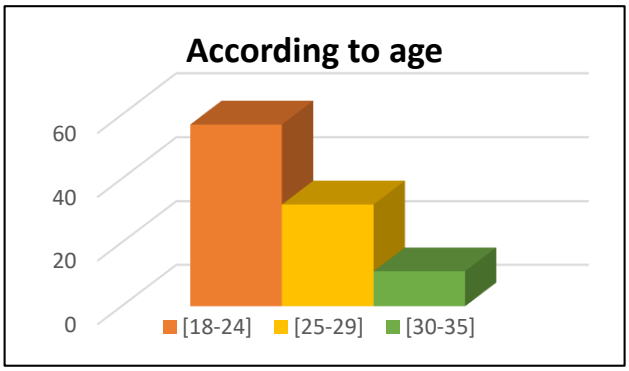

Figure 20: Subject's distribution according to age

1.1.2. According to family situation

This distribution allowed us to collect varied information for each subject, among the whole population, we noted 89% of single persons while only 11% were married (Figure 21).

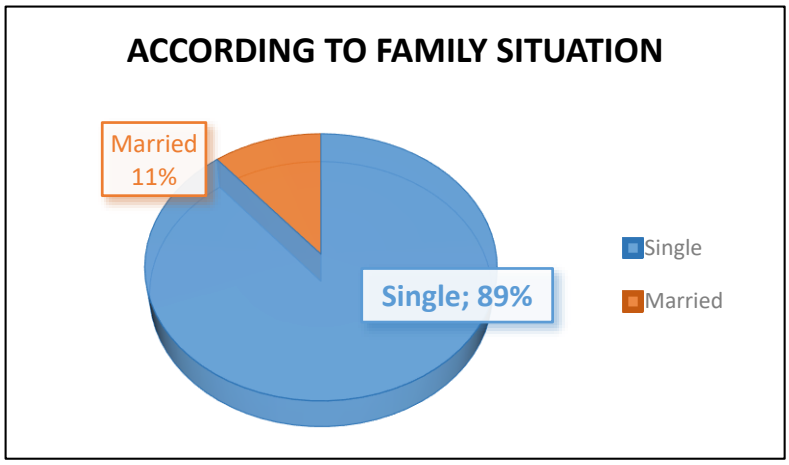

Figure 21: Subject's distribution according to family situation

1.1.3. According to educational state and occupation

88% of subjects of the study have a university educational level while only 06% with a high school level, 05% abandoned their studies in middle school and only 01% with a primary educational level (Figure 22). They occupy different professions, the majority of them (54%) are students, 13% are employees in state companies and 11% in private sector companies, while 22% are unemployed (Figure23).

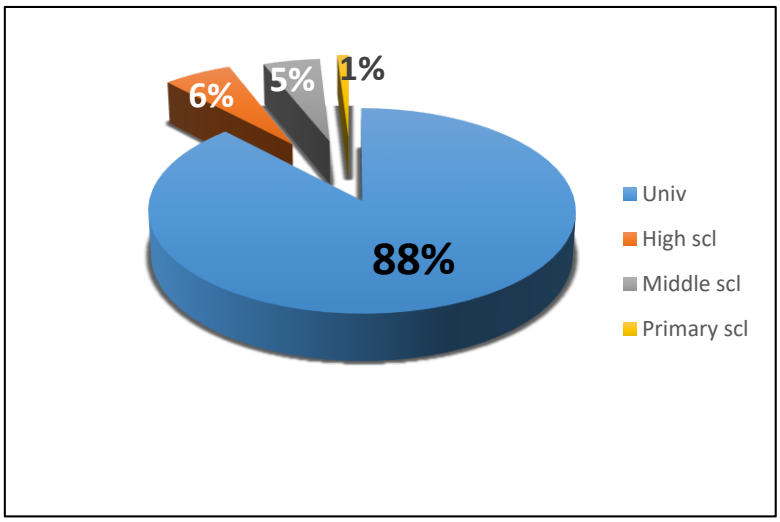

Figure 22: Subject's distribution according to educational status

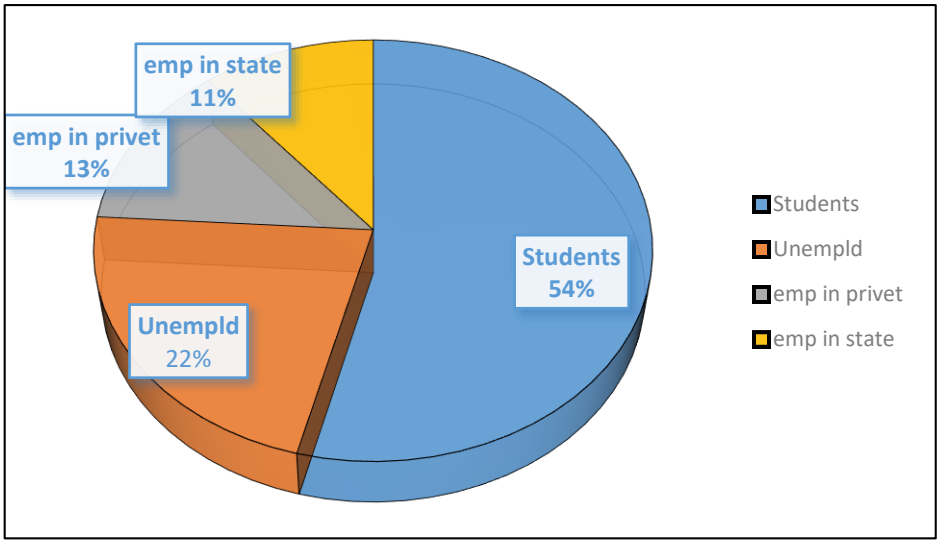

Figure 23: Subject's distribution according to occupation

1.2.Distribution according to clinical characteristics

1.2.1. According to BMI

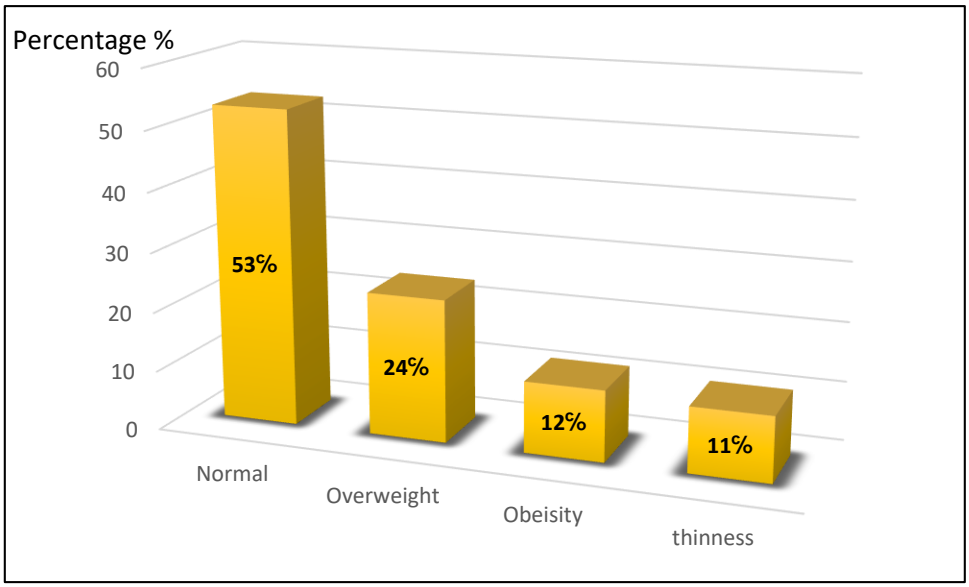

Figure 24: Subject's distribution according to BMI

According to World Health Organization (WHO,2016), BMI's interpretation follows intervals, a normal BMI is situated between 18.5 and 24.9, a BMI belongs to the interval 25 to 30 indicates an overweight, more than 30 means we are facing an obesity unlike a BMI less than 18.5 which indicates that the subject is too thin [09] Our study's results are represented in the graphic below (Figure 24). 53% persons have a normal BMI while 24% are classed in the overweight class. The subjects of the study that have a BMI more than 30 are 12% and they represent the obesity class. In a study of a group composed of 236 university students, the body height, weight, BMI, and other parameters were estimated. The diagnosis of obesity among participants according to BMI level has shown that 20.3% of subjects were overweight and 5.1% obese. With increasing BMI values (Gažarová *et al.*, 2019).

1.2.2. According to systolic and diastolic blood pressure

According to WHO,2013, Standards of systolic blood pressure value is 115 to 120 mmHg, concerning the diastolic blood pressure is 75 to 80 mmHg [10] The following graphics demonstrate our population's results (Figure 25 and 26).

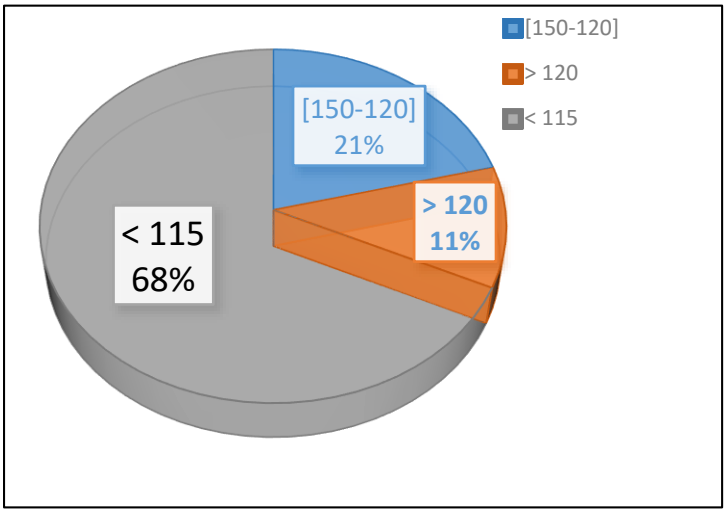

Figure 25: Subject's distribution according to systolic blood pressure

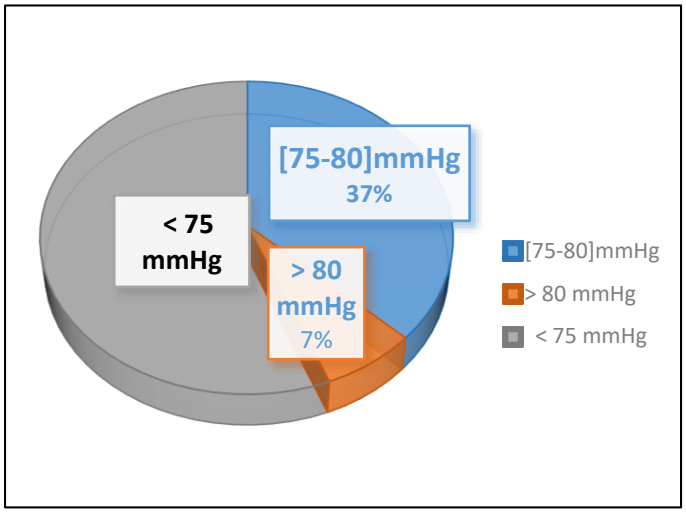

Figure 26: Subject's distribution according to diastolic blood pressure

1.2.3. According to smoking state

The survey's responses provided to us an information about the smoking status of our young population in which we identified that 91% of the population never smoke, this can be due to the fact the majority of population are women knowing that in Algerian society a women' smoking is unacceptable behavior. Among men, we found 2% that stopped smoking three years ago and 7% of them are actually smoking, results are detailed in the table below (Table 07).

Table 07: Subject's distribution according to smoking state

	Smokers subjects				
	Duration		Cigarette's number per day		
	1-10 years	>10 years	01 -10	10-30	> 30
Number of subjects	02	05	02	04	01
Percentage	28.57%	71,42%	28.57%	57,14%	14.28%

1.2.4. According to nutritional state

Since that the daily water and dairy products consumption has an important effect on various body functions, essentially on kidneys besides the crucial role that can play in the estimation of certain biological markers levels especially those used to evaluate a possible CKD among the studied population, the questionnaire has included questions about nutritional fact of these young subjects, the results are presented in the graphics below (Figures 27 and 28).

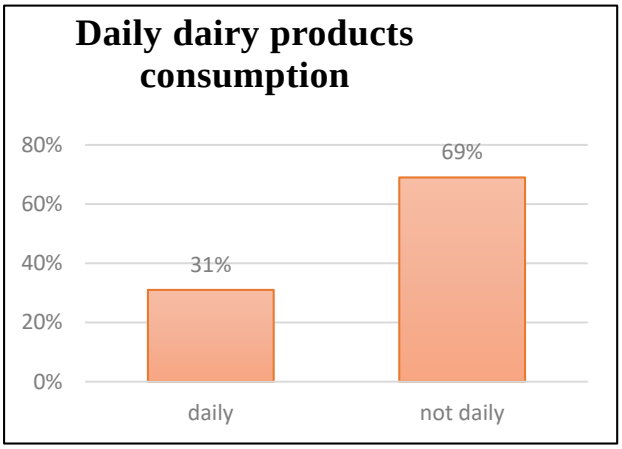

Figure 27: Daily dairy products consumption

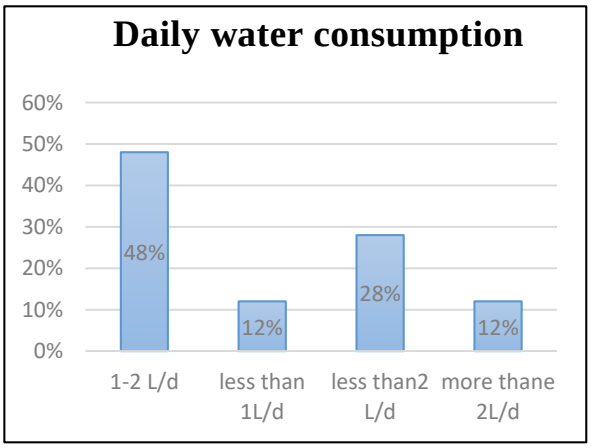

Figure 28: Daily water consumption

1.3.Distribution according to biological characteristics

1.3.1. Fasting blood glucose

Among the 100 persons that we measured their fasting blood glucose, 15% of them presents a fairly high ratios comparing to the limits: (0.68 - 1.10g/L) according to (Tchuem Tchuenté, 2021), the values of which vary from 1 g/L to 1.09m/L at the max. 80% (12 persons) of them are aged 20 to 25years, the 20% (3 persons) others are 26, 27 and 32 years. On the basis of BMI, we distinguish 26.6% (4 persons) with an overweight and 2.6% (4 others) that present an obesity. It is required to mention that 14% of the total population present DT2 in their medical family's history.

A person whose age is 23 years, with a blood pressure [150mmgh/78mmgh], used to smoke during 7 years with 3 cigarettes per day, he has stopped smoking three years ago, presents a fasting blood glucose limit value (1.10g/L) which is detected during the screening, a person whose age is 19 years whose blood pressure is [130mmHg/70mmHg], exposed to a negative smoke, with hyperglycaemia (1.22g/L) is also determinate. Both, they present an obesity state, sedentary physical activity besides the presence of DT2 in their medical family's history.

The conclusion here is that these 14 subjects who have an impaired fasting blood glucose and high BMI have prediabetes and must be confirmed with the measure of glycated hemoglobin (HbA1c). American diabetes Association (ADA) definitions for prediabetes include impaired fasting glucose (IFG) = fasting glucose 5.6–6.9 mmol/L and impaired glucose tolerance (IGT) = 2 h glucose 7.8–11.0 mmol/L. ADA has subsequently recommended (HbA1c) levels between 5.7% and 6.4% to define prediabetes (ADA, 2020).

Table 08: Fasting blood glucose result's identification

Blood fasting glucose (g/L)	**[1-1.09]**		**1.10**	**1.22**
Percentage(%)	80		1	1
Age (years)	[20-25]	[26-32]	23	19
	80%	20%		
BMI	overweight	Obesity	Obesity	Obesity
	26.6%	26.6%		
Smoking state	Majority never smoke		Stopped since 3 years	Exposed to negative smoke
Med family history	T2D close/distant		T2D close/distant	T2D distant
Physical activity	Majority walking 30min/ day		Sedentary	Sedentary

It is a matter of fact that predisposing factors for T2D are hypertension, dyslipidemia, smoking, and obesity. An obese 18-year-old has a greater than 50% risk of developing T2D and the higher his BMI the higher the risk increases. Actually, approximately 80% of T2D patients are obese or overweight (Gléau, 2021).

A T2D screening realized by Zeghri and his team in 2017, on a population of patients in which the age range is between 8 months to 80 years, The evaluation of overweight and obesity was carried out by the calculation of the BMI (BMI=Weight/Size2 (Kg/m2)), they are defined respectively by BMI > 25 Kg/m2, and BMI > 30 Kg/m2, weight and height were measured according to the recommendations of the WHO, The glycaemic control was carried out by blood analysis of HBa1c and fasting blood glucose. Standards are 7% for HBa1c and 0.70g/L to 1.10g/L for fasting blood glucose. The results showed that the overweight affects the entire

population with dominance of diabetes, Mean women's BMI tends towards obesity (BMI=29.15 Kg/m^2) for type 2 diabetes. The glycaemic control values are above the standards: with 8.5% > 7% for HBa1c and 1.3 > 1.10g/L for fasting glucose. This result can be explained by the fact that overweight can lead in most cases to a T2D (Zeghri et al., 2017)

Several studies showed that the increase in the prevalence of obesity explains the elevation in T2D. It is even called "diabesity". Obesity and T2D have a common denominator: unhealthy food associated with sedentary physical activities. The number of diabetic patients is actually expected to double to reach more than 350 million people worldwide by 2030, according to the who, This recently prompted a major awareness-raising campaign by the International Diabetes Federation and the International Association for the Study of Obesity (Rorive *et al*., 2005).

To explain how overweight and obesity can provoke T2D, the more fats are accumulated within the body, the more insulin the body needs. Which reduce the pancreas function that can't produce enough insulin to satisfy the body need, leading to a T2D development. In obese or overweight people, diabetes is therefore the consequence of being overweight (Mimita *et al*., 2018).

The hereditary side plays a crucial role in T2D's appearance, the lifetime risk of developing T2D is 40% for individuals who have one parent with T2D and 70% if both parents are affected. First degree relatives of individuals with T2D are about 3 times more likely to develop the disease than individuals without a positive family history of the disease, genes like TCF7L2 have been replicated in multiple studies, however how these genes interact with each other and with the environment to produce T2D is still poorly understood (Ali, 2013; Queitsch *et al*., 2012).

Comparing our research's results with these different studies, we infer that the 15% with a fairly high ratios of fasting blood glucose (1-1.09g/L) besides the one with a limit fasting blood glucose (1.10g/L) and the other with hyperglycaemia (1.22g/L) dispose more

risks factors of prediabetes such us overweight or obesity, hereditary T2D…etc which makes them more susceptible to be diagnosed with T2D few years later.

1.3.2. Blood creatinine and urea dosage

Following a plasma urine and creatinine assay, different values were recorded but remained within the standard interval for urea [0.15 to 0.45g/L] for creatinine [07 -14 mg/L] for men and [06 -11mg/L] for women (Landry *et al.*,2020; Delanaye *et al*., 2017).

- **Blood urea**

Normal values of blood urea: 0,15-0.24 g/L (according to the laboratory normal values)

From the 100 subjects of our the study, 66% them their plasma urea level is between the normal range [0.15 and 0.24 g/L], 27% of cases over than 0.24 g/L, while 07% of the population their plasma urea level is less than 0.15g/L (Table 09).

Table 09: Classification of the studied persons according To their blood urea levels

	Number of subjects	**Percentage**
Normal range [0.15 and 0.24 g/L]	66	66%
Low (less than 0.15 g/L)	07	07%
High (more than 0.24g/L)	27	27%

For the subject with 0.39 g/L urea value, it was found that he consumes a lot of dairy products (every day), with an acceptable water consumption with an average of 1 to 2 liters per day, we also noted an excessive consumption of nonsteroidal anti-inflammatory drug (NSAIDs).

Concerning the subject with the lowest urea value (0.11 g/L) the data of the questionnaire indicate a reduced consumption of the dairy products and absence of NSAIDs's or any other medication use comparing.

27% of cases are under medication: NSAIDs, Paracetamol, corticotherapy, 29.6% use tow or the three medications during the same period of treatment. From 27%, 66.6% present a blood urea level in between [0.20-0.39 g/L], while 33.3%, their blood urea level is less than 0.20 g/L.

From the 29.6% with combination of medications, 22.21% present a blood urea level in between [0.20-0.39 g/L] and the 7.40% have a blood urea level is less than 0.20 g/L (Table 10).

Table 10: Effect of NSAID/Corticoid/Paracetamol treatment on blood urea level

Blood urea values	**20-39 g/L**	**Less than 20g/L**
With only one drug (27%) (NSAID/CORTH/PARA)	66.6%	33.3%
With combined medications (NSAID/CORTH/PARA) (29.6%)	22.21%	7.40%

In conformity with the survey investigation, we found that more than 50% of cases with a urea blood values in between 0.20 and 0.39 g/L presents a daily water consumption vary from 1L-2lL/day to more than 2L/day, while those with lower blood urea (0.15 to 0.20 g/L and less than 0.15g/L) presents in general a poor dairy production consumption with water consumption less than 1L/day. However we faced multiple cases their blood values are in between 0.20 and 0.39g/L that don't consume an important quantity of dairy products and with a low daily consumption of water(less than o1L/day), Others with lower blood urea (0.15 to 0.20 g/L and less than 0.15g/L) show a contrary an important daily dairy products consumption with high daily water consumption which indicate that those factors can fluctuate considerably from person to another.

To better understand those results, we refer to several studies that have performed on urea dosage indicate that the plasmatic urea most of the time is influenced by a set of factors such as the amount of proteins in the daily diet or that is intake a night before the dosage: seen that the urea is the last product of proteins metabolism, a phenomena that takes place in the liver named urea cycle. Hepatocytes process toxic ammonia produced upon the metabolism of proteins and amino acids through a series of biochemical reactions that yield urea, a disposable by-product that

is excreted in the urine, if there is a lot of protein to be metabolized, more urea will reach the kidneys to be eliminated from the body, this will increase its concentration in the blood, blood urea level is affected also by the body hydration/ dehydration knowing that water is an essential molecule for well renal function, gastrointestinal hemorrhage besides some medications like corticosteroids (Hong *et al*,2023; Keshet *et al*., 2018; Wang *et al*.,2014). As an example of medication's affect DSAID are also involved, their common mode of action is to decrease prostanoid production by inhibiting the activity of the two cyclooxygenase isoforms (COX-1 and COX-2). COX-2 is an isoform expressed primarily during an inflammatory process, COX-1 is involved in the regulation of multiple physiological functions. The two main pathways lead to a renal cytoprotective vasodilatation, COX-2 pathway enhances sodium and water secretion, the inhibition of Cox-1 provoke an increase in modularly blood flow which conduct to a rise in natruiresis and decreasing in sodium retention consequently a low blood pressure will appear . While, COX-2 inhibition pathway is manifested by a decrease in modularly blood flow that cause a sodium level elevation and natruiresis decreasing, that will enhance the augmentation of blood pressure level, this two phenomena conduct to a renal dysfunction (Drożdżal *et al*.,2021).

Studies has shown that blood urea level depends enormously on multiple factors, of which these factors do not influence all people in the same way, the same factor therefore its impact is capable of being different from one person to another (Wang *et al*.,2014). That's explain the variation in our study results.

We may deduce then that blood urea level is not an exact marker by itself to diagnose a CDK, it is rather an excellent indicator that guides us to the performance of extra analysis with higher occurrence.

- **Blood creatinine**

Creatinine is formed by spontaneous and relatively constant conversion of creatine and phosphocreatine, which provides a rapid but short-term source of ATP in muscle and other tissues. It is a waste product of the body that comes from the breakdown of muscle's creatine. Creatinine's elimination is carried up by the renal filtration. When kidney's ability of eliminating waists decreases, the amount of creatinine increases in the blood. Its dosage in the blood therefore depends on kidney function but also on muscle mass**.** Blood creatinine is actually measured to

calculate the GFR level that we use to evaluate the quality of the renal function (Moore et Sharer, 2017; Hafeez *et al*.,2016).

Our study's results has shown a variation in blood creatinine level of the population but always within normal limits, accept one case in which we noted a low value 5.8 mg/L. Studies showed that a low blood creatinine level may be the sign of a low muscle mass, dystrophy or even a liver damage (Diag et Señaris, 2020).

- **Creatinine Clearance (MDRD)**

One of the measures of kidney function is the GFR. GFR describes the flow rate of filtered fluid through the kidney. The creatinine clearance rate (C_{Cr} or CrCl) is the volume of blood plasma that is cleared of creatinine per unit time and is a useful measure for approximating the GFR. The MDRD equation is often used to estimate the GFR. Several factors can impact the variation in MDRD values in CKD, including the progression of the chronic kidney disease, levels of creatinine in the blood, age, gender, dietary differences, and muscle mass (whether it is high or very low) (Lesley *et al*.,2014)

Through our study of the results of 100 persons, initially considered to be overall healthy, we found upon closer examination that they could be divided into two groups: The first group classified as "normal" and the 2nd group classified as "slightly low kidney function" (Table 11).

Table 11: Classification of the study subjects according to MDRD results

GFR(ml/min/1,73 m^2)	**CKD stage**	**Definition**	**Percentage**
[90 – 136]	1	GFR Normal	59 %
[66.9 – 90]	2	GFR slightly low	41 %

This distinction arises from variations in kidney function, where individuals in the first group, individuals show a high level of kidney efficiency, which is indicative of good kidney

function. This suggests that they may have a favourable prognosis. However, in the second group, individuals may not be fully aware of the seriousness of chronic kidney disease and its implications. It is important to emphasize the significance of CKD in this group and the need for appropriate management and monitoring to prevent further complications.

- **CKD Stage 1**: In this stage, the GFR is considered normal or only slightly decreased. The estimated GFR is ≥ 90 mL/min/1.73 m². Stage 1 kidney disease indicates very mild kidney damage and minimal loss of kidney function. At this stage, there may be signs of kidney damage, such as proteinuria (presence of protein in the urine) or abnormal imaging results. However, the overall kidney function is still normal or near normal.
- **CKD Stage 2**: In stage 2, the GFR is mildly decreased. The estimated GFR is between [60-89] mL/min/1.73 m². Stage2 kidney disease signifies mild kidney damage and a slight reduction in kidney function. While the kidneys are not functioning at full capacity, the decline in GFR is still considered relatively mild. Similar to stage 1, there may be signs of kidney damage present (Lesley *et al*.,2014).

The disparity observed in stage 2 results we can be elucidated by the influence of specific medical conditions, namely diabetes, hypertension (high blood pressure), and autoimmune disorders, on renal function, consequently resulting in a diminished GFR. Furthermore, the administration of certain medications adversely affects kidney function and leads to a decline in GFR. Examples of such medications encompass certain antibiotics and non-steroidal anti-inflammatory drugs (NSAIDs). The observed outcomes may also be indicative of renal

damage or impairment, thereby contributing to the reduction in GFR values. Ultimately, other factors, such as variances in muscle mass and individual discrepancies in renal function, may also play a role in influencing GFR disparities (Hounkpatin *et al*.,2019).

It's important to note that the MDRD equation is an estimation tool that has its limitations. It may not be as accurate in certain populations, such as individuals with extremely high or low muscle mass, kidney transplant recipients, or those with acute kidney injury. For a comprehensive evaluation of kidney function, additional tests and clinical assessment by a healthcare professional are necessary (Salvador *et al.*, 2017).

1.3.3. Urine chemisery

The results of urine chemistry test are presented in the table below (Table 12).

Table 12: The results of urine chemistry test

<table>
<tr><td colspan="8">Results of the Urine chemistry</td></tr>
<tr><td>Normal</td><td colspan="7">Cases with problems detected</td></tr>
<tr><td>75%</td><td colspan="7">25%</td></tr>
<tr><td rowspan="6"></td><td colspan="7">problems detected</td></tr>
<tr><td colspan="3">Blood</td><td>Proteins</td><td colspan="2">Leukocytes</td><td rowspan="3">Nitrites</td></tr>
<tr><td colspan="3">18</td><td>12</td><td colspan="2">5</td></tr>
<tr><td colspan="3">66.6%</td><td>44.4%</td><td colspan="2">18.5%</td></tr>
<tr><td>trails</td><td>(+)</td><td>(++)</td><td>Trails</td><td>(+)</td><td>(++)</td><td>Positive</td></tr>
<tr><td>04</td><td>07</td><td>07</td><td>12</td><td>04</td><td>01</td><td>01</td></tr>
<tr><td>22.22%</td><td>38.88%</td><td>38.88%</td><td>100 %</td><td>80%</td><td>20%</td><td>3.7%</td></tr>
</table>

In our study, 66.6% of the subjects have a blood in their urine a phenomenea that named hematuria. Hematuria is one of the alarming manifestations of a renal disease. Two hematuria types are distinguished: macroscopic hematuria and microscopic hematuria. It can be the result of a urinary infection or a renal dysfunction, in most of cases the microscopic haematuria doesn't indicate serious complications yet, it can be the sign of an alteration in kidney's function. Renal hematuria causes are multiple, they can be due to glomerular or non-glomerular diseases (Figure 30) (Madaan *et al*., 2022; Vedula et Iyengar, 2020).

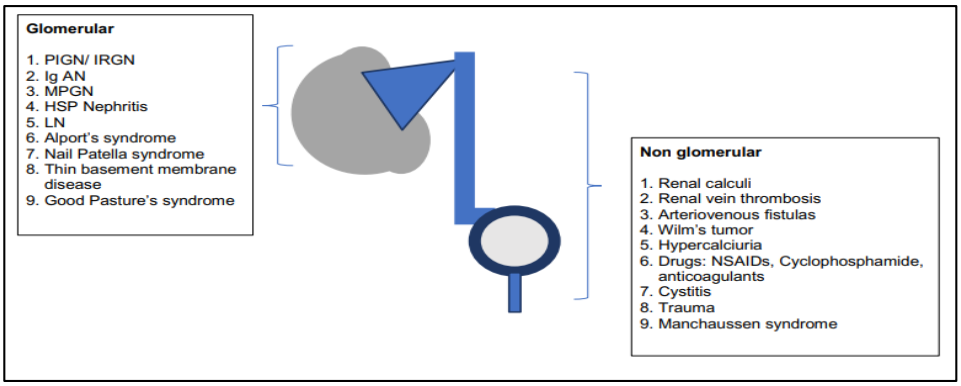

Figure 29: Glomerular and non-glomerular hematuria causes
(Vedula et Iyengar, 2020)

The urine chemistry test reveals 44.4% of the population with proteins trails, the presence of proteins trails in urine is actually common, it can be the result of physiological state. Physiological proteinuria is made up approximately half of proteins produced and secreted by the tubule and the urothelium: Tamm Horsfall protein (uromodulin), urokinase, secretory IgA, however albumin is the main protein observed in the urine, but always in quantity lower than 15-30 mg/24 hours in physiological situation. There are well-identified situations where proteinuria is transient, unrelated to a structural anomaly of the glomerular filtration barrier: orthostatic proteinuria, intensive physical effort and feverish state. To prevent the loss of serum proteins during the filtration process, the kidney relies on the complex structure of the glomerular filtration barrier (GFB), supplemented by reuptake of filtered proteins by the renal tubules, in Pathologic proteinuria the alterations at GFB level hinder the filtration of proteins which will stop its reabsorption in the blood leading to its accumulation in urine, witch explain the abnormal level of proteins level in urine. Proteinuria in majority of time is an indication of a renal complication just

like CKD, but sometimes it can indicate also a urinary infection or diabetes (Sharma et Smyth, 2021; Pallet *et al*., 2019). In our study, the person with high fasting blood glucose (1.22g/L), protein and blood trails are identified in its urine chemistry. Actually an important part of those presenting blood or proteins in urines, their fasting blood glucose level is more than 01g/L.

The detection of leukocytes and nitrites in urines indicate a urinary viral or bacterial infection. During the infection within the body, immune system will response starting the inflammation process which involves several inflammation's mediators besides the immune cells that we call leukocytes, leukocytes that increased their number within the blood in order to defend the body against the pathogenic will then be eliminated throw the urines (Abdulkhaleq *et al*.,2018; Hicklinget *al*., 2015). Positive nitrite in urines is also an indicator of bacterial infection, Nitrite is a toxic molecule resulting from the conversion of a Nitrate (NO3) non-toxic molecule present naturally in the urine tracts to toxic Nitrite NO^{2-} by a Gram negative bacteria (Kalugalage *et al.*, 2013*).*

Finally, our results indicate that the population studied of young peoples on which we performed our screening tests had identified 14 cases of suspected prediabetes and 41% of the population with CKD stage 2 that require a close monitoring of the renal function. In addition to this, an important part of the subjects have serious risk factors that make them susceptible to develop more serious complications in the future. Extra tests are indispensable to confirm our results such us: HbA1C, impaired glucose tolerance, microalbuminuria, and 24H proteinuria.

Conclusion

Conclusion

CKD is a serious medical condition characterized by a gradual loss of kidney's function over the time, it is defined us the presence, for more than 3 months, of renal damage's markers and a drop in the GFR's level less than 60 ml/min/1.73m2. Diabetes and high blood pressure, or hypertension, are the main causes of CKD. Several risk factors are involved in enhancing the development of CKD such us age, obesity, smoking state, genetic factors and many others. The evaluation of the kidney's function is made through the estimation of the GFR using MDRD equation.

Diabetes is a common condition that affects people of all ages. There are several forms of diabetes. Type 2 is the most common, patients with T2D present essentially two problems: The pancreas does not produce enough insulin — a hormone that regulates the movement of glucose into the cells or cells respond poorly to the insulin produced which augment the amount of glucose in the blood.

This two diseases (CKD and T2D) are characterized by silent evolution to the final stage. In this present study, our main objective is to detect the prevalence of CKD as well as prediabetes among normally healthy young subjects (18-35 years old) and to sensitize them to the particularity and severity of these diseases.

This study was carried out on a group of 100 persons aged (18-35years old) divided between 17% and 83% men and women respectively. Our investigation is based on a questionnaire intended for each person followed by biological tests of fasting blood glucose, blood urea and creatinine and urine chemistry test. The study focused also on certain factors such as obesity, and high blood glucose, family medical history, blood pressure which are the main risk factors in our diseases. Other factors were also studied such as daily diet, smoking and others.

Our results indicate that the population studied of young peoples on which we performed our screening tests had identified 14 cases of suspected prediabetes and 41% of the population with CKD stage 2 that require a close monitoring of the renal function. In addition to these, an important part of the subjects have serious risk factors that make them susceptible to develop more serious complications in the future. Extra tests are indispensable to confirm our results such us: HbA1C, impaired glucose tolerance, microalbuminuria, and 24H protein

Perspectives and limits of the study

In this study, an increase of the number of the subjects screened for more than 1000 cases would be more significant, and more types of lab tests must be programed to be performed such as lipid profile, HbA1C, proteinuria and microalbuminuria, since the biological tests already done during our investigation are the primitive tests and must be confirmed. Due to the lack of time and financial resources, unfortunately we were not able to carry it out.

The research work was normally done at the 8 Mai 1945 university with only students and university staff. Obstacles prevent us from doing so. What actually makes us encounter problems in the collection of volunteers, in particular with the lack of people' awareness about the scientific research spirit or even the fear of discovering any disease.

For these reasons we are convinced that our research deserves possible prosecution. To this end, we propose to deepen this study by:

- Performing more accurate analyses for those who presented risk of CKD or prediabetes.
- Launch national mass screening company with the large possible number of young people aged from 18 to 35 years.

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List of attachments

Attachment 01: the questionnaire

1- Identification du sujet :

Nom :…… **Age** : …….

Prénom :…… **N° Téléphone** : …….

GENRE : H ☐ F ☐

2- Caractéristiques sociologiques :

- **Situation familiale:** Marié(e) ☐ Célibataire ☐ Divorcé(e) ☐ veuf (ve) ☐
- **Profession** :
  - **Haut fonctionnaire de l'état** ☐
  - **Employé de l'état** ☐
  - **Haut cadre dans le privé** ☐
  - **Employé dans le privé** ☐
  - **indépendant(e)** ☐
  - **Ouvrier (e)** ☐
  - **Etudiant, lycéen(e)** ☐
  - **Femme au foyer** ☐
  - **Retraité(e)** ☐
  - **Chômeur (se)** ☐
  - **Invalide** ☐
- **Niveau d'instruction :**
  - **Aucune instruction officielle** ☐
  - **Ecole primaire** ☐
  - **Collège** ☐
  - **Lycée ou équivalent** ☐
  - **Ecole supérieur, Université** ☐
  - **Diplôme universitaire** ☐

3- Paramètres anthropométriques :

- **Poids**: ……Kg **Taille:** …….cm
- **IMC**: ……kg/cm^2
- **Tour de taille** : ……cm
- **Pression artérielle systolique :**
- **Pression artérielle diastolique :**

4- Etat tabagique :

Fumeurs ? Non, jamais ☐

Non, j'ai arrêté depuis moins de 3ans ☐

Non, j'ai arrêté depuis plus de 3 ans ☐

Oui ☐ Quantité (en nombre de cigarette/jr)……

Depuis combien d'années ? ……

5- Activité physique

Habitudes : sédentaire ☐ sport (2à 3 fois/sem) ☐ marche 30min/jr ☐

6- Les habitudes alimentaires :

- Consommation d'eau : Moins de 1litre/j ☐
  1-2 litres /j ☐
  Plus de 2litres/j ☐
- Consommation de boissons sucrées : Tous les jours ☐ Pas tous les jours ☐
- Consommation des produits laitiers : Tous les jours ☐ Pas tous les jours ☐
- Consommation des légumes et fruits: Tous les jours ☐ Pas tous les jours ☐

7- Les antécédents médicaux personnels :

- Hyperglycémie : oui non
- HTA oui non
- DYSTHYROIDIE oui non
- HEPATITE VIRALE oui non
- Pathologie rénale oui non
- Lithiase rénale oui non
- HEMOGLOBINOPATHIE oui non
- Pancréatite oui non
- Chirurgicales oui non
- Autres …….

8- Les antécédents familiaux :

- **Diabète :**

☐ Non

☐ Oui, un membre de la famille plus éloignée : un grands-parents, une tante, un oncle, un(e) cousin(e).

☐ Oui, un membre de la famille proche : un père, une mère, un enfant, un frère, une soeur...

- HTA oui non
- DYSTHYROIDIE oui non
- HEPATITE VIRALE oui non

- Pathologie rénale oui non
- Lithiase rénale oui non
- HEMOGLOBINOPATHIE oui non

9- <u>Prise de médicaments : (ultérieure ou en cours)</u>

- Antihypertenseurs oui non
- AINS oui non
- Corticothérapie oui non
- Autres ……..

Consentement éclairé

Je, soussigné…… déclare accepter, librement, et de façon éclairer, de participer comme sujet à l'étude intitulée :……..
J'autorise Dr ……à :

- Effectuer des dosages sanguins et urinaires
- Recueillir les données personnelles

L'enquêteur principal s'engage à préserver absolument la confidentialité et le secret professionnel pour toutes les informations concernant le participant et mener cette recherche selon les dispositions éthiques et déontologiques.

Le consentement pour poursuivre la recherche peut être retiré à tout moment sans donner de raison et sans encourir aucune responsabilité ni conséquence.

Fait à le……..

ttachment 02: Fasting blood glucose dosage

SPINREACT

CE GLUCOSE -TR

Glucose-TR

Trinder. GOD-POD

Quantitative determination of glucose
IVD

Store at 2-8°C

PRINCIPLE OF THE METHOD

Glucose oxidase (GOD) catalyses the oxidation of glucose to gluconic acid. The formed hydrogen peroxide (H_2O_2), is detected by a chromogenic oxygen acceptor, phenol-aminophenazone in the presence of peroxidase (POD):

$$\beta\text{-D-Glucose} + O_2 + H_2O \xrightarrow{GOD} \text{Gluconic acid} + H_2O_2$$

$$H_2O_2 + \text{Phenol} + \text{Aminophenazone} \xrightarrow{POD} \text{Quinone} + H_2O$$

The intensity of the color formed is proportional to the glucose concentration in the sample[1,2].

CLINICAL SIGNIFICANCE

Glucose is a major source of energy for most cells of the body; insulin facilitates glucose entry into the cells.
Diabetes is a disease manifested by hyperglycemia; patients with diabetes demonstrate an inability to produce insulin[1,5,6].
Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

REAGENTS

R 1 Buffer	TRIS pH 7,4	92 mmol/L
	Phenol	0,3 mmol/L
R 2 Enzymes	Glucose oxidase (GOD)	15000 U/L
	Peroxidase (POD)	1000 U/L
	4 - Aminophenazone (4-AP)	2,6 mmol/L
GLUCOSE CAL	Glucose aqueous primary standard	100 mg/dL

PREPARATION

Working reagent (WR): Dissolve (→) the contents of one vial R 2 Enzymes in one bottle of R 1 Buffer.
Cap and mix gently to dissolve contents.
The reagent is stable 1 month after reconstitution in the refrigerator (2-8°C) or 7 days at room temperature (15-25°C).

STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8°C, protected from light and contaminations prevented during their use.
Do not use reagents over the expiration date.
Signs of reagent deterioration:
- Presence of particles and turbidity.
- Blank absorbance (A) at 505 nm ≥ 0,10.

ADDITIONAL EQUIPMENT

- Spectrophotometer or colorimeter measuring at 505 nm.
- Matched cuvettes 1,0 cm light path.
- General laboratory equipment.

SAMPLES

Serum or plasma, free of hemolysis[3] and CSF.
Serum should be removed from the clot as quickly as possible.
Stability: Glucose is stable at 2-8°C for 3 days.

PROCEDURE

1. Assay conditions:
   Wavelength: ..... 505 nm (490-550)
   Cuvette: ..... 1 cm light path
   Temperature: ..... 37°C / 15-25°C
2. Adjust the instrument to zero with distilled water.
3. Pipette into a cuvette[Note 3]:

	Blank	Standard	Sample
WR (mL)	1,0	1,0	1,0
Standard[Note 1,2] (µL)	--	10	--
Sample (µL)	--	--	10

4. Mix and incubate for 10 min at 37°C or 20 min at room temperature (15-25°C).
5. Read the absorbance (A) of the samples and standard, against the Blank. The colour is stable for at least 30 minutes.

CALCULATIONS

$$\frac{\text{(A) Sample - (A) Blank}}{\text{(A) Standard - (A) Blank}} \times 100\ (\text{Standard conc.}) = \text{mg/dL glucose in the sample}$$

Conversion factor: mg/dL x 0,0555= mmol/L.

QUALITY CONTROL

Control sera are recommended to monitor the performance of assay procedures: SPINTROL H Normal and Pathologic (Ref. 1002120 and 1002210).
If control values are found outside the defined range, check the instrument, reagents and calibrator for problems.
Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES[1]

Serum or plasma:
60 – 110 mg/dL ≅ 3,33 – 6,10 mmol/L
CSF:
60 – 80% of the blood value

These values are for orientation purpose; each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS

Measuring range: From *detection limit* of 0,000 mg/dL to *linearity limit* of 500 mg/dL.
If the results obtained were greater than linearity limit, dilute the sample 1/2 with NaCl 9 g/L and multiply the result by 2.
Precision:

	Intra-assay (n=20)		Inter-assay (n=20)	
Mean (mg/dL)	91,9	249	93,2	250
SD	0,49	1,28	1,35	2,78
CV (%)	0,54	0,52	1,45	1,11

Sensitivity: 1 mg/dL = 0,0331A.
Accuracy: Results obtained using SPINREACT reagents (y) did not show systematic differences when compared with other commercial reagents (x).
The results obtained using 50 samples were the following:
Correlation coefficient $(r)^2$: 0,99812.
Regression equation: y= 1,1405x -2,5580.
The results of the performance characteristics depend on the analyzer used.

INTERFERENCES

Haemoglobin up to 4 g/L, bilirubin up to 20 mg/L, creatinine up to 100 mg/L and galactose up to 1g/L do not interfere.
A list of drugs and other interfering substances with glucose determination has been reported[3,4].

NOTES

1. GLUCOSE CAL: Proceed carefully with this product because due its nature it can get contaminated easily.
2. Calibration with the aqueous standard may cause a systematic error in automatic procedures. In these cases, it is recommended to use a serum Calibrator.
3. Use clean disposable pipette tips for its dispensation.
4. **SPINREACT has instruction sheets for several automatic analyzers. Instructions for many of them are available on request.**

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PACKAGING

Ref: 1001190	Cont.	R1: 4 x 125 mL, R2: 4 → 125 mL, CAL: 1 x 5 mL
Ref: 1001191		R1: 4 x 250 mL, R2: 4 → 250 mL, CAL: 1 x 5 mL
Ref: 1001192		R1: 10 x 50 mL, R2: 10 → 50 mL, CAL: 1 x 5 mL

BSIS17-I 12/05/16

SPINREACT, S.A./S.A.U. Ctra.Santa Coloma, 7 E-17176 SANT ESTEVE DE BAS (GI) SPAIN
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Attachment 03: Blood creatinine dosage

SPINREACT

CE CREATININE -J

Creatinine

Jaffé. Colorimetric - kinetic

Quantitative determination of creatinine
IVD

Store at 2-8°C

PRINCIPLE OF THE METHOD

The assay is based on the reaction of creatinine with sodium picrate as described by Jaffé.
Creatinine reacts with alkaline picrate forming a red complex. The time interval chosen for measurements avoids interferences from other serum constituents.
The intensity of the color formed is proportional to the creatinine concentration in the sample[1].

CLINICAL SIGNIFICANCE

Creatinine is the result of the degradation of the creatine, component of muscles, it can be transformed into ATP, that is a source of high energy for the cells. The creatinine production depends on the modification of the muscular mass, and it varies little and the levels usually are very stable.
Is excreted by the kidneys. With progressive renal insufficiency there is retention in blood of urea, creatinine and uric acid.
Elevate creatinine level may be indicative of renal insufficiency[1,2].
Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

REAGENTS

R 1 Picric Reagent	Picnic acid	17,5 mmol/L
R 2 Alkaline Reagent	Sodium hydroxide	0,29 mol/L
CREATININE CAL	Creatinine aqueous primary standard	2 mg/dL

PRECAUTIONS

R1/ R2: H314-Causes severe skin burns and eye damage.
CAL: H290-May be corrosive to metals.
Follow the precautionary statements given in MSDS and label of the product.

PREPARATION

Working reagent (WR):
Mix equal volumes of R1 Picric Reagent and R2 Alkaline reagent.
The working reagent is stable for 15 days at 2-8°C or 7 days at room temperature (15-25°C).

STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8°C, protected from light and contaminations prevented during their use.
Do not use reagents over the expiration date.
Signs of reagent deterioration:
- Presence of particles and turbidity.
- Blank absorbance (A) at 492 nm ≥ 1,80.

ADDITIONAL EQUIPMENT

- Spectrophotometer or colorimeter measuring at 492 nm (490-510).
- Matched cuvettes 1,0 cm light path.
- General laboratory equipment.

SAMPLES

- Serum or heparinized plasma[1].
  Creatinine stability: 24 hours at 2-8°C.
- Urine (24 h): Dilute sample 1/50 with distilled water. Mix. Multiply results by 50 (dilution factor);
  Creatinine stability: 7 days at 2-8°C.

PROCEDURE

1. Assay conditions:
   Wavelength: ..... 492 nm (490-510)
   Cuvette: ..... 1 cm light path
   Temperature: ..... 37°C / 15-25°C
2. Adjust the instrument to zero with distilled water.
3. Pipette into a cuvette (Note 3).

	Blank	Standard	Sample
WR (mL)	1,0	1,0	1,0
Standard (Note 1,2,3) (µL)	--	100	--
Sample (µL)	--	--	100

4. Mix and start stopwatch.
5. Read the absorbance (A_1) after 30 seconds and after 90 seconds (A_2) of the sample addition.
6. Calculate: $\Delta A = A_2 - A_1$.

CALCULATIONS

$$\frac{\Delta A \text{ Sample} - \Delta A \text{ Blank}}{\Delta A \text{ Standard} - \Delta A \text{ Blank}} \times 2 \text{ (Standard conc.)} = \text{mg/dL of creatinine in the sample}$$

Conversion factor: mg/dL x 88,4 = µmol/L.

QUALITY CONTROL

Control sera are recommended to monitor the performance of assay procedures: SPINTROL H Normal and Pathologic (Ref. 1002120 and 1002210).
If control values are found outside the defined range, check the instrument, reagents and calibrator for problems.
Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES[1]

Serum or plasma:

Male	0,7 - 1,4 mg/dL	≅ 61,8 - 123,7 µmol/L
Female	0,6 - 1,1 mg/dL	≅ 53,0 - 97,2 µmol/L

Urine: 15-25 mg/Kg/24 h

Male	10 - 20 mg/Kg/24 h
Female	8 - 18 mg/Kg/24 h

These values are for orientation purpose; each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS

Measuring range: From *detection limit* of 0,000 mg/dL to *linearity limit* of 35 mg/dL.
If the results obtained were greater than linearity limit, dilute the sample 1/2 with NACl 9 g/L and multiply the result by 2.
Precision:

	Intra-assay (n=20)		Inter-assay (n=20)	
Mean (mg/dL)	0,92	3,43	0,96	3,50
SD	0,03	0,07	0,04	0,09
CV (%)	2,76	1,90	3,97	2,51

Sensitivity: 1 mg/dL = 0,0407 ΔA/min.
Accuracy: Results obtained using SPINREACT reagents (y) did not show systematic differences when compared with other commercial reagents (x).
The results obtained using 50 samples were the following:
Correlation coefficient $(r)^2$: 0,99584.
Regression equation: y= 0,953x + 0,075.
The results of the performance characteristics depend on the analyzer used.

INTERFERENCES

Hemoglobin (1 g/L), Bilirrubin (55 mg/dL), interfere[1]. Lipids < 4 g/L do not interfere.
A list of drugs and other interfering substances with creatinine determination has been reported[2,3].

NOTES

1. CREATININE CAL: Proceed carefully with this product because due its nature it can get contamined easily.
2. Calibration with the aqueous Standard may cause a systematic error in automatic procedures. In these cases, it is recommended to use a serum Calibrator.
3. Use clean disposable pipette tips for its dispensation.
4. **SPINREACT has instruction sheets for several automatic analyzers.**

BIBLIOGRAPHY

1. Murray R.L. Creatinine. Kaplan A et al. Clin Chem The C.V. Mosby Co. St Louis. Toronto. Princeton 1984; 1261-1266 and 418.
2. Young DS. Effects of drugs on Clinical Lab. Tests, 4th ed AACC Press, 1995.
3. Young DS. Effects of disease on Clinical Lab. Tests, 4th ed AACC 2001.
4. Burtis A et al. Tietz Textbook of Clinical Chemistry, 3rd ed AACC 1999.
5. Tietz N W et al. Clinical Guide to Laboratory Tests, 3rd ed AACC 1995.

PACKAGING

Ref: 1001110	Cont.	R1: 1 x 50 mL, R2: 1 x 50 mL, CAL: 1 x 2 mL
Ref: 1001111		R1: 1 x 150 mL, R2: 1 x 150 mL, CAL: 1 x 5 mL
Ref: 1001112		R1: 1 x 1000 mL, R2: 1 x 1000 mL, CAL: 1 x 5 mL
Ref: 1001113		R1: 2 x 250 mL, R2: 2 x 250 mL, CAL: 1 x 5 mL

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Attachment 04: Blood urea dosage

SPINREACT

CE UREA -B

Urea-B

Berthelot. Enzymatic colorimetric

Quantitative determination of urea

IVD

Store at 2-8°C

PRINCIPLE OF THE METHOD

Urea in the sample is hydrolized enzymatically into ammonia (NH_4^+) and carbon dioxide (CO_2).

Ammonia ions formed reacts with salicylate and hypochlorite (NaClO), in presence of the catalyst nitroprusside, to form a green indophenol:

$$Urea + H_2O \xrightarrow{Urease} (NH_4^+)_2 + CO_2$$

$$NH_4^+ + Salicylate + NaClO \xrightarrow{Nitroprusside} Indophenol$$

The intensity of the color formed is proportional to the urea concentration in the sample[1,2,3].

CLINICAL SIGNIFICANCE

Urea is the final result of the metabolism of proteins; it is formed in the liver from its destruction.

Elevated urea can appear in blood (uremia) in: diets with excess of proteins, renal diseases, heart failure, gastrointestinal hemorrhage, dehydration or renal obstruction[1,6,7].

Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

REAGENTS

R 1 Buffer	Phosphate pH 6,7	50 mmol/L
	EDTA	2 mmol/L
	Sodium salicylate	400 mmol/L
	Sodium nitroprusside	10 mmol/L
R 2 NaClO	Sodium hypochlorite (NaClO)	140 mmol/L
	Sodium hydroxide	150 mmol/L
R 3 Enzymes	Urease	30000 U/L
UREA CAL	Urea aqueous primary standard	50 mg/dL

PRECAUTIONS

R2: H314-Causes severe skin burns and eye damage.

Follow the precautionary statements given in MSDS and label of the product.

PREPARATION

- Working reagent (WR): Dissolve (→) one tablet R 3 Enzymes in one bottle of R 1 Buffer. Cap and mix gently to dissolve contents.
  Stability: 4 weeks in the refrigerator (2-8°C) or 7 days at room temperature (15-25°C).
- R 2 NaClO is ready to use.

STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8°C, protected from light and contaminations prevented during their use.

Do not use reagents over the expiration date.

Do not use the tablets if appears broken.

Signs of reagent deterioration:

- Presence of particles and turbidity.
- Blank absorbance (A) at 580 nm ≥ 0,32.

ADDITIONAL EQUIPMENT

- Spectrophotometer or colorimeter measuring at 580 nm.
- Matched cuvettes 1,0 cm light path.
- General laboratory equipment (Note 2).

SAMPLES

- Serum or heparinized plasma[1]: Do not use ammonium salts or fluoride as anticoagulants.
- Urine[1]: Dilute sample 1/50 in distilled water. Mix. Multiply results by 50 (dilution factor). Preserve urine samples at pH < 4.

Urea is stable at 2-8°C for 5 days;

PROCEDURE

1. Assay conditions:
   Wavelength: ..... 580 nm
   Cuvette: ..... 1 cm light path
   Temperature: ..... 37°C / 15-25°C
2. Adjust the instrument to zero with distilled water.
3. Pipette into a cuvette: (Note 4)

	Blank	Standard	Sample
WR (mL)	1,0	1,0	1,0
Standard(Note 1,3) (µL)	--	10	--
Sample (µL)	--	--	10

4. Mix and incubate 5 min at 37°C or 10 min at room temperature (15-25°C).
5. Pipette:

	Blank	Standard	Sample
R 2 (mL)	1,0	1,0	1,0

6. Mix and incubate 5 min at 37°C or 10 min at room temperature (15-25°C).
7. Read the absorbance (A) of the samples and calibrator, against the Blank. The colour is stable for at least 30 minutes at 15-25°C.

CALCULATIONS

$$\frac{(A)\ Sample - (A)\ Blank}{(A)\ Standard - (A)\ Blank} \times 50\ (Standard\ conc.) = mg/dL\ urea\ in\ the\ sample$$

mg/dL Urea x 0,466 = mg/dL Urea BUN (Blood Urea Nitrogen)[1].

Conversion factor: mg/dL x 0,1665 = mmol/L.

QUALITY CONTROL

Control sera are recommended to monitor the performance of assay procedures: SPINTROL H Normal and Pathologic (Ref. 1002120 and 1002210).

If control values are found outside the defined range, check the instrument, reagents and calibrator for problems.

Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES[1]

Serum : 15- 45 mg/dL (2,49-7,49 mmol/L)
Urine : 20 - 35 gr/24 h.

These values are for orientation purpose; each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS

Measuring range: From *detection limit* of 0,001mg/dL to *linearity limit* of 225 mg/dL.

If the results obtained were greater than linearity limit, dilute the sample 1/2 with NaCl 9 g/L and multiply the result by 2.

Precision:

	Intra-assay (n=20)		Inter-assay (n=20)	
Mean (mg/dL)	39	126	40,0	127
SD	0,55	2,12	0,93	2,48
CV (%)	1,43	1,68	2,33	1,96

Sensitivity: 1 mg/dL = 0,00608 A.

Accuracy: Results obtained using SPINREACT reagents (y) did not show systematic differences when compared with other commercial reagents (x).

The results obtained using 50 samples were the following:

Correlation coefficient $(r)^2$: 0,99143.

Regression equation: y= 1,0476x – 0,2846

The results of the performance characteristics depend on the analyzer used.

INTERFERENCES

It is recommended to use heparin as anticoagulant. Do not use ammonium salts or fluoride[1].

A list of drugs and other interfering substances with urea determination has been reported by Young et. al[4,5].

NOTES

1. UREA CAL: Proceed carefully with this product because due its nature it can get contaminated easily.
2. Glassware and distilled water must be free of ammonia and ammonium salts[1].
3. Calibration with the aqueous standard may cause a systematic error in automatic procedures. In these cases, it is recommended to use a serum Calibrator.
4. Use clean disposable pipette tips for its dispensation.
5. **SPINREACT has instruction sheets for several automatic analyzers.**

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1. Kaplan A. Urea. Kaplan A et al. Clin Chem The C.V. Mosby Co. St Louis. Toronto. Princeton 1984; 1257-1260 and 437 and 418.
2. Tabacco A et al. Cin Chem 1979; 25: 336-337.
3. Fawcett J K et al. J Clin Path 1960; 13: 156-169.
4. Young DS. Effects of drugs on Clinical Lab. Tests, 4th ed AACC Press, 1995.
5. Young DS. Effects of disease on Clinical Lab. Tests, 4th ed AACC 2001.
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7. Tietz N W et al. Clinical Guide to Laboratory Tests, 3rd ed AACC 1995.

PACKAGING

Ref: 1001331	Cont.	R1: 2 x 150 mL, R2: 2 x 150 mL, R3: 2 → 150 mL, CAL: 1 x 5 mL
Ref: 1001329		R1: 5 x 50 mL, R2: 5 x 50 mL, R3: 5 → 50 mL, CAL: 1 x 5 mL

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