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RESEARCH ARTICLE

DNA- Drug Binding and Uric Acid Concentrations Relationship in Renal Failure

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Abstract

Kidney diseases are recognised as a significant health problem affecting approximately 188 million peoples in low- and middle-income countries. In this study, investigation of the relationship between DNA and UA concentrations and the interaction of DNA and drug in renal failure patients was studied. DNA was extracted from serum and whole blood by two methods, kit and manual to find the best way, by comparison, the results. Nanodrop and electrical migration technique were used to measure the obtained concentration of DNA. The interaction between DNA and drug (Allopurinol) in the presence of UA was done by using ultraviolet and infrared spectroscopies. The study included 80 healthy and 80 patients samples with chronic renal impairment with ages rang (20-80) of both genders. The result showed a significant increase in the concentration of UA, urea and creatinine in renal failure patients compared to control a significant reduction in DNA concentration in patients with high UA concentration, the DNA extracted by manual method was higher compared to the results of the kit. The type of interference between DNA and Allopurinol is intercalative binding.

Keywords: *Kidney disease, Allopurinol, DNA, Uric acid and renal failure.*

Introduction

Renal failure is a disorder in which the kidneys fail to remove metabolic end-products from the blood and regulate the fluid, electrolyte, and pH balance of the extracellular fluids. Renal failure can occur as two types, acute kidney disease (AKD) and chronic kidney disease (CKD) [1]. The causes of AKD commonly are categorized as pre-renal (55% to 60%), intrinsic or intrarenal (35% to 40%) and postrenal (<5%), post renal impaired exudation caused by cardiogenic shock and heart failure and decreased vascular filling because of increased vascular capacity (e.g., anaphylaxis or sepsis).

Aged individuals are especially at risk because of their inclination to hypovolemia and their high predominance of renal vascular disorders [2].Several studies have demonstrated a link between hyperuricemia and gout. The risk for crystal formation increases as increasing of urate concentration, which may increase the patient's susceptibility to the development of gout [3].It has been estimated that 20 to 60% of sufferers with gout also have mild renal dysfunction, showing a probable link between an elevated UA level and renal disease [4]. Although hyperuricemia may represent a marker of renal disease, there are some studies suggest that elevated UA levels might contribute to the development and progression of renal failure [5]. One of the possible mechanisms by which UA may worsen the progression of renal disease is through the activation of the renin-angiotensin system (RAS). The RAS has been documented as a contributor to the progression of the renal disease by inducing fibrosis of renal and vascular cells or by increasing both systemic and glomerular pressure [6].

The treatment strategies for deficient renal function in uremic patients involve dialysis therapies. One of the possible consequences in the dialysis process is oxidative stress, which can result in damage to the DNA [7, 8]. Likewise, oxidative stress seems to be a primary harmful effect resulted from increased UA. Also, it was found that high levels of UA in the renal cortex of

experimental animals have a decreasing effect on mitochondrial DNA (mtDNA). Therefore, correcting serum UA levels has become a common strategy in the treatment of many diseases. One modality in the treatment of gout, for example, is to reduce the overall serum UA concentration, which is either accomplished through dietary and lifestyle changes or treatment with UA reducing drugs such as Allopurinol or Febuxostat [9, 10]. Transcription and replication are vital to cell survival and proliferation as well as for smooth functioning of all body processes. DNA starts transcribing or replicating only when it receives a signal, which is often in the form of a regulatory protein binding to a particular region of the DNA. Thus, if a small molecule can mimic the binding specificity and strength of this regulatory protein, then DNA function can be artificially modulated, inhibited or activated by binding this molecule instead of the protein [11]. Small molecules interact with DNA primarily by three binding modes electrostatic, groove and intercalative binding (Figure 1). [12] Drugs bind to DNA both covalently and non-covalently. Covalent binding in DNA is irreversible and invariably leads to complete inhibition of DNA processes and subsequent cell death. Non-covalent binding is reversible and is typically preferred over covalent adduct formation keeping the drug metabolism and toxic side effects in mind [11].

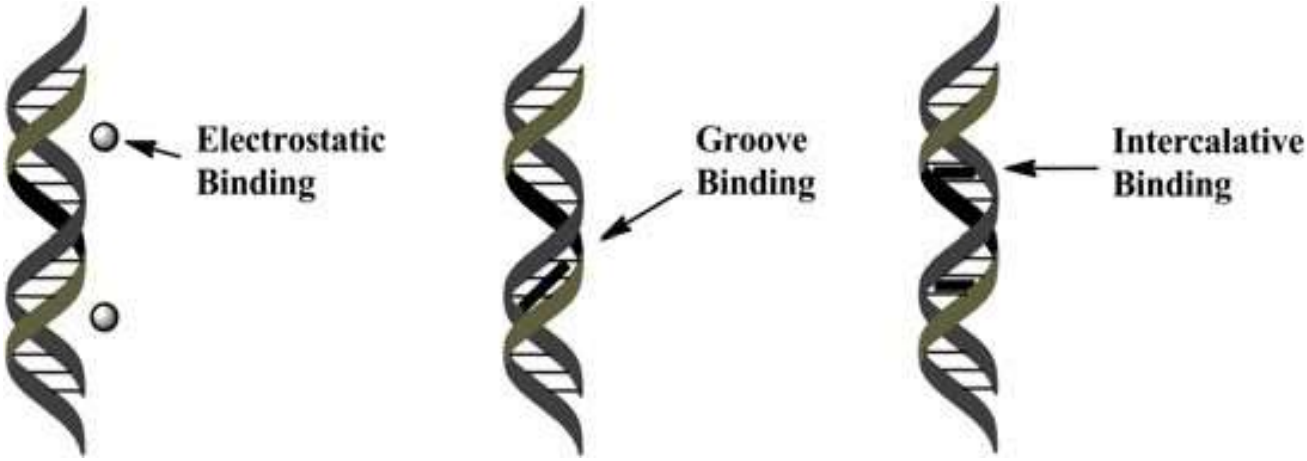

Figure 1: Design of electrostatic groove and intercalative binding of small molecules to the DNA

This study aimed to examine the integrity and concentration and biochemical parameters of patients with CKD. Further, to investigate the possibility of the direct action of Gout drugs on genomic DNA of same patients.

Materials and Methods

Blood Samples

Two and a half millilitres of venous blood was withdrawn by vein puncture using a syringe. The blood was separated in K_2EDTA tube left to clot at room temperature, and then the tube was centrifuged at (704Xg) for 10 mint, then serum was frozen at -20 ˚C until analysis.

The hemolyzed sample was discarded. Also for DNA extraction, the whole blood was entirely used. The samples were collected from Kirkuk General Hospital- artificial kidney Department / Iraq during the period from July 2016 to July 2017. They have been grouped into two sets as below:

First Group

Control group include (80) healthy peoples from (20-50) years and (50-80) years.

Second Group

Chronic kidney disease (CKD) group included (80) patients from (20-50) years and (50-80) years. Doctors have diagnosed the clinical status of patients specialised in artificial kidney department. All patients were subjected to personal interview using a specially designed questionnaire format to full history with detail information.

Methods

Estimation of Creatinine

Colourimetric reaction (Jaffe reaction) of creatinine with alkaline picrate measured kinetically at 490 nm (490-510) without any pretreat step. This reaction has been improved (specifically, speed and

adaptability) by the development of an initial-rate method [13, 14].

Reagents: Vial R1 BASE: Disodium phosphate (6.4 mmol/l) and Sodium hydroxide (150 mmol/l).

Vial R2 DYE: Sodium dodecyl sulfate (07.5 mmol/l) and Picric acid (4.0 mmol/l).

Viral R3 STANDARD: Creatinine 2mg/ dl (177 µmol/l)

The results have been calculated as follow:

$$Concentration\ of\ creatinine = (A2 - A1)ASSAY / (A2 - A1)standard \times standard\ concentration$$

Estimation of Blood Urea

Urea-kit enables endpoint enzymatic determination of urea concentrations (Urease- modified Berthelot reaction) in human urine serum or plasma.

Urease hydrolyses urea by producing ammonia:

In an alkaline medium, the ammonium ion reacts with the salicylate and hypochlorite to form a green coloured indophenol (2, 2 dicarboxylindophenol).

The colour intensity is proportional to the urea concentration in the sample [15, 16].

Reagents: Vial R1 Standard: Urea (8.33 mmol/l (0.5g/l).

Vial R2 Enzymes: Urease (≥ 350 Ku/l).

Vial R3 Color reagent: Phosphate buffer PH 8 (50 mmol/l), Sodiumsalicylate (62 mmol/l), Sodium nitropuusside (3.35 mmol/l)

EDTA(1 mmol/l). **Vial R4 Alkaline reagent:** Sodium hydroxide (NaOH) 0.5 mmol/l Sodium Hypochlorite (NaClO) 24.8 mmol/l.

Calculation

$$Concentration\ of\ Urea = Abs\ assay / Abs\ standard * standard\ concentration$$

Estimation of Uric Acid

Acid Unique Enzymatic PAPM Enables the Determination of UA using the uricase- peroxides- chromogen Sequence

The intensity of the measured colouration (quinoneimine) proportional to the quantity of uric acid in the sample.[17,18]

Reagents: Reagent 1 Standard: Uric acid 476 µmol/l or 80 mg/l,

Reagent 2 chromogen: buffer Tris buffer PH 8.0 50 mmol 3.5 dichloro -2 hydroxybenzene sulfonic acid surface- active agent.

Reagent 3: Uricase 80 U/l, Peroxide ≥ 200 U/l, Ascorbate oxidase ≥ 1000 U/l, 4-aminoantipyrine 0.25 mmol/l ,Potassium ferrocyanide 0.030 mmol. Wavelength 520 nm (510 to 546 nm)

Calculation

$$Concentration\ of\ sample = Asample / Astandard \times standard\ concentratio$$

DNA Extraction

Kit DNA Extraction

DNA extraction was determined by using (g SYNC)

Reagents: Wash buffer: add absolute ethanol then mix by shaking for a few seconds, **SB buffer**. **W1 buffer., GST buffer. Proteinase K:** add ddH2O (ph=7.0-8.5) then vortex to ensure Proteinase K is completely dissolved, centrifuge for a few seconds to spin down the mixture for extended periods, and they should be stored at 4°C. , **Elution buffer:** 10Mm tris-HCl (ph=8.5).

Blood Sample Preparation

Transfer up to 200 µl of whole blood, serum, plasma, buffy coat or body fluids to a 1.5 ml micro centrifuge tube. Adjust the volume to 200 µl with PBS. Add 20 µl of proteinase K then mix by pipetting. Incubate at 60 °C for 5 minutes.

Cell Lysis

Add 200 µl for GSB Buffer then mix by shaking vigorously. For blood and cell samples incubate at 60 °C for 5 minutes, inverting the tube every 2 minutes. For amniotic fluid at 60 °C for at least 20 minutes, inverting the tube every 5 minutes. During incubation, transfer required volume of elution buffer (200µl/sample) to a 1.5 micro centrifuge tube and heat to 60 °C.

DNA Binding

Add 200 µl of absolute ethanol to the sample lysate and mix immediately by shaking vigorously 10 seconds. If precipitate appears, break it up as much as possible with a pipette. Place a GS column in a 2 ml collection tube. Transfer all of the mixture (including any insoluble precipitate) to the GS column. Centrifuge at 14-16,000 x g for 1 minute. Following centrifugation, if the mixture did not flow through the GS column membrane, increase the centrifuge time until it passes completely. Discard the 2 ml collection tube containing the flow-through then transfer the GS column to a new 2 ml collection tube.

NOTE: it is crucial that the lysate and ethanol be mixed thoroughly to yield a homogenous solution.

Wash

Add 400 µl of W1 Buffer to the GS column. Centrifuge at 14-16,000 x g for 30 seconds then discard the flow- through. Place the GS column back in the 2 ml collection tube. Add 600 µl of Wash Buffer (make sure absolute ethanol was added) to the GS column. Centrifuge at 14-16,000 x g for 30 seconds then discard the flow-through. Place the GS column back in the 2 ml collection tube. Centrifuge again at 14-16,000 x g to dry the column matrix.

Elution

Transfer the dried GS column to a clean 1.5 ml micro centrifuge tube. Add 100 µl of pre-heated Elution Buffer, TE Buffer or water into the CENTER of the column matrix. Let stand for at least 3 minutes to allow Elution Buffer, TE Buffer or water to be completely absorbed. Centrifuge at 14-16,000 x g for 30 seconds to elute purified DNA.

Manual DNA Extraction

Fast and inexpensive DNA extraction protocol from whole blood then was an essential step to follow up a reaction in genetic engineering and molecular-based methods.[19,20]

Reagents

Buffer a Solution (Red Blood Cell Lysis Buffer)

- 10 mMTris-HCl: dissolve 1.21g tris base 0.1M in100ml dH2O and 38.5ml 0.1N Hcl with 11.5 dH2o to complete volume to 50ml., then mix tris base with Hcl.
- 0.32 M sucrose: dissolve 5.4g sucrose in 50 ml dH2o.
- 0.75% Titon-X- 100: mix 0.75ml Titon-X-100 with 99.25ml ddH2o.
- 5 mM MgCl2 (PH 7.6): dissolve 0.002 g MgCl2 in 50 ml dH2o.

All contents of Buffer A were mixed and adjusted Ph to7.6.

Buffer B Solution (White Blood Cell lysis Buffer)

- 4Mm Na2EDTA: dissolve 0.07g Na2EDTA 0.004M in 50ml dH2o.
- 20 mMTris-HCl.
- 100nMNaCl: dissolve 0.58g NaCl 0.1M in 100ml dH2o.

All contents of Buffer B were mixed and adjusted Ph to 7.4.

SDS10%: dissolve 10g SDS in 80ml dH2o.

Procedure

- The blood sample was collected using venipuncture from a healthy volunteer and added into tubes containing Ethylene diamine tetraacetic acid (EDTA).
- One millilitre of fresh or frozen (thawed at room temperature) whole blood was transferred into 2 ml micro-tube and mixed into 1 ml of distilled water.
- Micro-tube was mixed for 30 sec and then was centrifuged at 7000 rpm for 5 min. The

supernatant was discarded and repeated step ii and iii for 2 times.

NOTE: in each time the pellet was break up and mixed by shaking and vortexing.

- One ml of buffer A was added to the pellet and again mixed with vortex for the breakup and rinse.
- It was centrifuged at 7000 rpm for 5 min, and the supernatant was removed.

NOTE: if pellet has red blood cells, step 4 and 5 was repeated.

- In this step, one ml of buffer B and 100 µl of SDS 10% were added, and the pellet was suspended with vortexing and or shaking, then tube incubated in 65 °C for 15 min.
- Micro tube chilled with ice for 3 min, and 400 µl of saturated NaCl (5M) was added and gently was shaken for 30 sec and centrifuged at 5000 rpm for 10 min at 4 °C.
- About one ml of the upper phases transferred into a new 2 ml tube, an equal volume of absolute cold ethanol was added.
- Gently shacked the tube; in this step, DNA appeared as a white skein.
- Again tube was centrifuged at 13000 rpm for 10 min at 4 °C.
- The supernatant was discarded, and the pellet was washed with 1 ml of chilled 70% ethanol and centrifuged again at 10000 rpm for 10 min.
- The supernatant was discarded, and the pellet was left at room temperature to be dried.
- 50-100 microliter of ddH2O was added to dissolve the pellet and DNA solution stored at 20 °C until use.

Note that for more yielded DNA, we can use more volume of blood, up to 5 ml, in a bigger tube such as 15 ml falcon; all the remaining procedure is the same.

Measurement of DNA Concentration

Measurement of DNA Concentration from Whole Blood and Serum by Nanophotometer

Nanophotometer technology is based on an innovative sample retention system that uses the surface tension to hold and measure micro volume samples between two optical pedestals without the use of cuvettes or capillaries.

The Nanophotometer used to quickly and easily measure 0.5-2 µL droplets of proteins, DNA, RNA, and other biomolecules [21].

Agarose Gel Electrophoresis for Samples

Agarose gel electrophoresis is the most effective way of separating DNA fragments of varying sizes ranging from 100 bp to 25 kb [22].

Reagents

- Agarose solutions.
- 0.07%Ethidium bromide.
- Electrophoresis buffer.
- DNA Ladder.

DNA Binding

In Vitro Binding DNA with UA

Reagents

- Tris-HCl (PH=7.4): dissolve 1.21g tris base 0.1M in100ml dH2O and 38.5ml 0.1N HCl with 11.5 dH2o to complete volume to 50ml.
- KOH solution: dissolve 0.06g KOH in 10ml dH2O.
- DNA solution was prepared and that by taking 10 ml of human DNA and putting it in tris-HCl of (PH= 7.4).

In Vitro Binding DNA with Allopurinol (Zyloric) (Z)

Reagent

Tris-HCl (PH=7.4), DMSO, DNA solution, Benedict's reagent, H2SO4 and Alfa naphthol.

In Vitro Binding DNA with Uric Acid and Allopurinol (Zyloric) (UAZ)

The dissolved substances were mixed in their different solvents and treated as a single solution and were added according to the previous steps

Results and Discussion

This study included Eighty samples of blood with renal failure and 80 as healthy subjects with age ranged (20-80) years. That patient has visited the Kirkuk General Hospital-artificial kidney department and biochemical variables measured in serum Blood in the control group and patient according to the methods of work described in Chapter II.

Studied groups were divided by gender and age as shown in Table (1).

Table 1: Shows the concentration (mg/dl) of urea, uric acid and creatinine of the patients and controls with the male gender

Mean ± St. De						
Control creatinine (mg/dl)	Creatinine (mg/dl)	Control Urea (mg/dl)	Urea (mg/dl)	Control Uric acid (mg/dl)	Uric acid (mg/dl)	Age
0.931 ± 0.323	8.641 ± 3.865	37.926 ± 9.595	122.158 ± 34.039	5.115 ± 1.029	10.955 ± 5.74	20-50
0.974 ± 0.452	6.648 ± 2.949	37.688 ± 12.557	123.208 ± 25.118	5.713 ±1.002	9.140 ± 2.208	50-80

Table 2: Shows the concentration (mg/dl) of urea, uric acid and creatinine of the patients and controls with the female gender

Mean ± St. De						
Control creatinine (mg/dl)	Creatinine (mg/dl)	Control Urea (mg/dl)	Urea (mg/dl)	Control Uric acid (mg/dl)	Uric acid (mg/dl)	Age
0.869±0.338	7.437 ± 1.674	28.111±5.839	121.011 ± 30.5	3.956 ±1.007	8.449 ± 2.833	20-50
0.915±0.426	7.041 ± 2.704	37.579±16.787	135.888 ± 38.998	4.845±1.55	9.771 ± 3.82	50-80

The results showed that there was a significant increase (P≤0.001) in the concentration of the creatinine in patients with CKD for male of group (20-50) years is (8.641 ± 3.865) and of group (50-80) years is (6.648 ± 2.949) respectively compared with control is (0.931 ± 0.323) and (0.974 ± 0.452) , while the same concentration for female of group (20-50) years is equal to (7.437 ± 1.674) and of group (50-80) years is (7.041 ± 2.704) compared with control (0.869 ± 0.338) and (0.915 ± 0.426) as shown in (Table1,2)

The high concentration of creatinine in serum CKD may be due to the fact that creatinine is a metabolic waste that is naturally expelled by urine and in the case of kidney failure renal insufficiency prevents it from filtering and excreting that due the high concentration of creatinine in serum and its concentration is inversely proportional to The speed of glomerular filtration with a slight decrease in GFR increases the concentration of creatinine in the blood [23, 24].

The results showed that there was a significant increase (P≤0.001) in the concentration of the urea in patients with CKD for male of group (20-50) years is (122.158 ± 34.039) and of group (50-80) years is (123.208 ± 25.118) respectively compared with control is (37.926 ± 9.595) and (37.688 ± 12.557) while the same concentration for female of group (20-50) years is equal to (121.011 ± 30.5) and of group (50-80) years is (135.888 ± 38.998) compared with control (28.111±5.839) and (37.579±16.787).

The reason of high urea concentration in the blood of CKD patients may be due to the fact that urea is the basic material of nitrogen from metabolic wastes which consists mainly in the liver and put out through the urine and in the case of kidney failure occurrence gets defect and a decrease in kidney function which leads urea to collect and accumulate in the blood and increases its focus which reflects the defect in the function filtration for college and the speed of urea formation, this increase depends on the severity and progress of renal deficit and to have proteins and to speed up catabolism rate. Results in (table1,2) showed a significant increase (p≤0.001) in the concentration of uric acid in patients with CKD for male of group (20-50) years is (10.955 ± 5.74) and of group (50-80) years is (9.140 ± 2.208) respectively compared with control is (5.115 ± 1.029) and (5.713 ±1.002) while the same concentration for female of group (20-50) years is equal to (8.449 ± 2.833) and of group (50-80) years is (9.771 ± 3.82) compared with control (3.956 ±1.007) and (4.845±1.55).

The increase in uric acid concentration in patients with CKD caused hyperuricemia. These results are in agreement with that of Roncal and others (2007) who reported that hyperuricemia is known to cause acute renal failure via intrarenal crystal deposition [25].

Extraction of DNA by Kit and Manual Methods

In this study, two methods were used to extract DNA (kit and manual) from serum and whole blood. When the two methods were compared it turned out that commercial DNA isolation kits are fast and safe, but are usually very expensive and low yielded. Common salting out method is laborious and time consuming. Therefore, optimized and efficient DNA extraction protocol is an essential step to follow up reaction in genetic engineering and molecular based methods [26, 27].And the kit method receiving to high yielded, inexpensive, simple and also rapid protocol without using enzyme reagent (Figure 3).

Showed the result of DNA electrophoresis for kit and manual samples for whole blood .

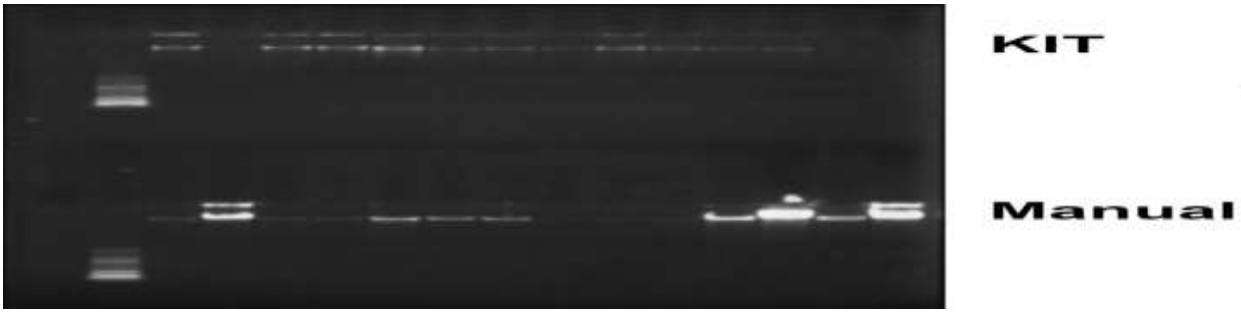

Figure 2: Shows the results of DNA concentration by electrophoresis for the kit and manual samples of whole blood

The results showed that the DNA concentration was inversely proportional to the uric acid concentration as shown in the table where the (8kit) has a high concentration of uric acid but the concentration of DNA is very low, and (1kit) has a low concentration of uric acid but the concentration of DNA is high .The purity of the DNA was assayed with spectrophotometric analysis that showed the A260/A280 ratio of (1.8) to (2).It is possible that a uric acid anion is also formed through the reaction of uric acid with hydroxyl radical .the anion is known to inactivate certain enzymes and may therefore be capable of DNA damage [28].

Table3: Shows DNA concentration by a kit for whole blood, and the results were taken from the NanoPhotometer

#	Name	Conc.	Units	A230	A260	A280	A320	A260/A280	A260/A230	Dilution
1	Blank 1	0.0000	ng/ul	0.000	0.000	0.000	0.000	0.000	0.000	
2	1 kit	71.250	ng/ul	4.487	2.005	1.601	0.580	1.396	0.365	15
4	2 kit	4.8000	ng/ul	0.081	0.075	0.024	-0.021	2.133	0.941	15
5	3 kit	29.600	ng/ul	0.274	0.571	0.281	-0.021	1.960	2.007	15
6	4 kit	34.000	ng/ul	0.308	0.655	0.332	-0.025	1.905	2.042	15
7	5 kit	47.400	ng/ul	1.713	0.871	0.537	-0.077	1.544	0.530	15
8	6 kit	10.500	ng/ul	0.041	0.159	0.060	-0.051	1.892	2.283	15
9	7 kit	33.350	ng/ul	0.608	0.645	0.506	-0.022	1.263	1.059	15
10	8 kit	1.7000	ng/ul	0.003	-0.006	-0.017	-0.040	1.478	0.791	15
11	9 kit	55.700	ng/ul	3.501	1.071	0.853	-0.043	1.243	0.314	15
12	10 kit	38.550	ng/ul	3.037	0.694	0.583	-0.077	1.168	0.248	15
13	11 kit	101.60	ng/ul	10.01	2.630	2.674	0.598	0.979	0.216	15
14	12 kit	8.8000	ng/ul	0.303	0.120	0.053	-0.056	1.615	0.490	15
15	13 kit	2.4000	ng/ul	0.110	0.044	0.022	-0.004	1.846	0.421	15

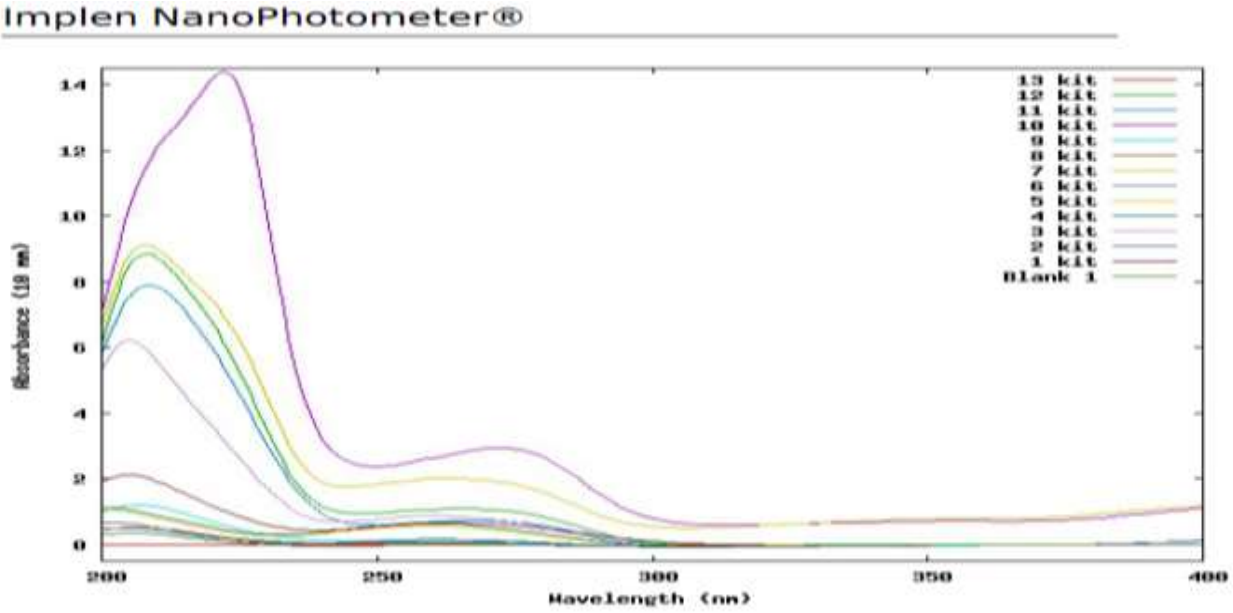

Figure 3: Shows DNA concentration by a kit for whole blood, and the results were taken from the NanoPhotometer

Table 4: Shows Manual results of DNA concentration for whole blood

#	Name	Conc.	Units	A230	A260	A280	A320	A260/A280	A260/A230	Dilution
16	Blank 2	0.0000	ng/ul	0.000	0.000	0.000	0.000	0.000	0.000	
17	1 M	6.5500	ng/ul	0.093	0.130	0.068	-0.001	1.899	1.394	15
18	2 M	181.40	ng/ul	1.597	3.630	1.887	0.002	1.925	2.275	15
19	3 M	5.3500	ng/ul	0.143	0.105	0.066	-0.002	1.574	0.738	15
20	4 M	4.1000	ng/ul	0.063	0.076	0.032	-0.006	2.158	1.188	15
21	5 M	24.650	ng/ul	0.338	0.491	0.261	-0.002	1.875	1.450	15
22	6 M	12.350	ng/ul	0.145	0.250	0.125	0.003	2.025	1.739	15
23	7 M	10.100	ng/ul	0.107	0.200	0.103	-0.002	1.924	1.853	15
24	8 M	0.5000	ng/ul	-0.053	-0.008	-0.010	-0.018	1.250	-0.286	15
25	9 M	2.4000	ng/ul	0.052	0.048	0.033	-0.000	1.455	0.923	15
26	10 M	2.4500	ng/ul	0.039	0.047	0.027	-0.002	1.690	1.195	15
27	11 M	25.750	ng/ul	0.271	0.516	0.271	0.001	1.907	1.907	15
28	12 M	20.550	ng/ul	0.313	0.409	0.214	-0.002	1.903	1.305	15
29	13 M	303.90	ng/ul	2.695	6.084	3.164	0.006	1.925	2.260	15
30	14 M	229.55	ng/ul	2.058	4.597	2.391	0.006	1.925	2.237	15

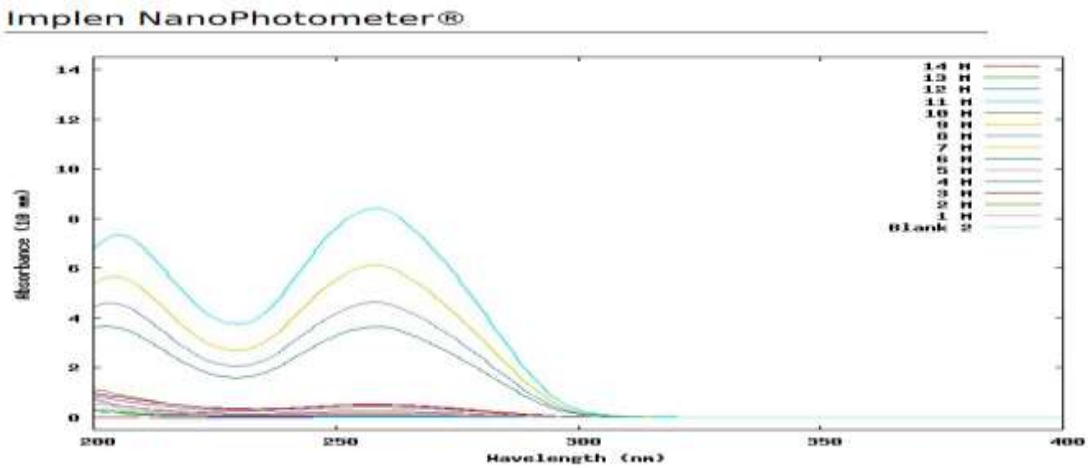

Figure4: Shows Manual results of DNA concentration for whole blood

The results as shown in (Table 4) that the concentration of DNA is inversely proportional to the concentration of uric acid as in (13M) ,(14M) With a normal concentration of uric acid while the concentration of DNA is high whereas the concentration of DNA is low in all other samples with high uric acid concentration.
UV spectroscopy was used to study the drug interaction with DNA and UA. In the pectra of DNA with varying concentration of UA and Zyloric. There are four major bands in the spectra for pure DNA, 213 nm (negative), 205nm (positive), 231nm (negative), 255nm (negative) and 264nm (positive).

These are marker UV bands of double helical DNA in ß-conformation [29]. UV–visible spectra of free DNA and Zyloric and UA-DNA complexes are shown in the table (3-6). Increase in the intensity of absorption was observed for all Zyloric & uric acid-DNA complexes as compared to free DNA. The proportional increase in the absorption intensity is seen with increasing concentration of Zyloric and UA in Zyloric and UA-DNA complexes. This hyper chromic effect indicates toward intercalative binding of the Zyloric & uric acid-DNA which might results in extension, unwinding and stiffness of DNA helix.[30,31]This unwinding exhibits increased exposure of nitrogenous bases which in turn causes an increase in absorption maxima (A260) of DNA. The absorption maxima of Zyloric & uric acid-DNA complexes also shift towards the longer wavelength as compared to free DNA. This bathochromic shift (red shift) is also an indication of the formation of an intercalating complex between Zyloric & uric acid and DNA.

This shift can be attributed to the less polar environment encountered by the drug molecules in the hydrophobic core of the DNA molecule upon the formation of Zyloric & uric acid DNA complex.[32] Binding constant was calculated determine the stability of the (DNA+Uric acid -Zyloric) complex using the method described earlier. The double reciprocal plot of $1/ (A-A_o)$ versus $1/C_z$ is show in (figure 5).The binding constant (K) can be estimated by calculation the ratio of the intercept to the stop [33].

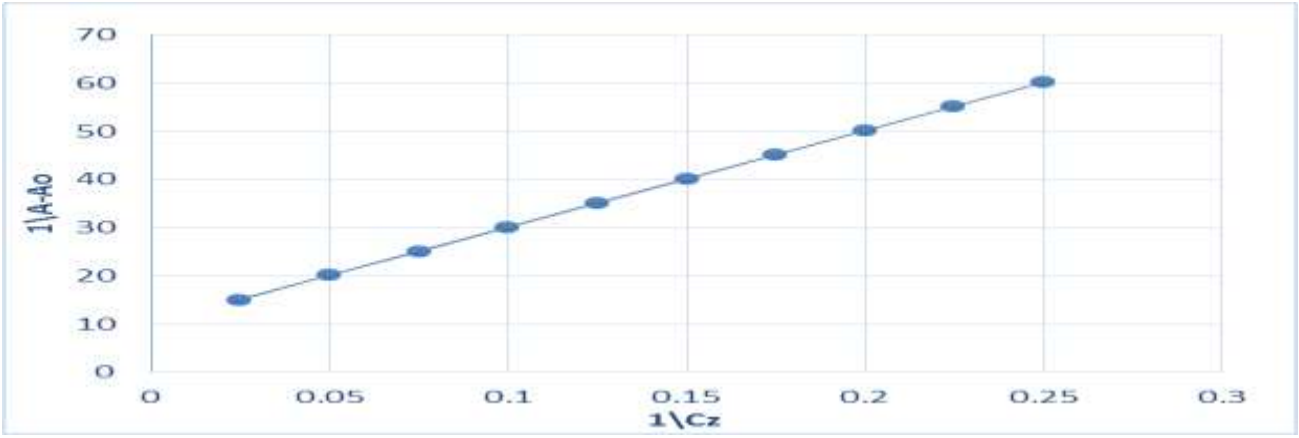

Figure 5: Double reciprocal plot between 1\A-A$_o$ and 1\C$_z$
A_o is the initial absorption of free DNA
A is the absorption at different concentration at 260nm
C_z is the analytical concentration of Zyloric in solution

Table 5: Shows UV–visible spectra of free DNA and zyloric & uric acid-DNA complexes

DNA and derivatives	Absorption A	Binding constant K	Type of transition
Free DNA	0.030	2.1×10^3	Hyper shift
DNA+ Uric acid	0.06	4.3×10^3	Hyper shift
DNA+ Zyloric	0.25	5.1×10^3	Hyper shift
DNA+ Uric acid & Zyloric	0.31	7.1×10^3	Red shift & Hyper shift

FTIR spectroscopy was also used to study the interaction. Free DNA show B conformation with infrared marker band at 1677 cm $^{-1}$ (Gua), 1226 cm $^{-1}$ (asymmetric Po2 stretch), 870 cm $^{-1}$ (sugar-phosphate stretch), and 807 cm $^{-1}$ (phosphodiester mode) [34].

FTIR spectra of free DNA and its complexes with different molar ratios of (uric acid), (Zyloric), and (uric acid & Zyloric) are shown in Figure 6,7and8 respectively. Peak assignments are by the literature. [35,36] Shifts are observed for many prominent IR bands of DNA when Zyloric & uric acid is added. Guanine band at 1713 cm $^{-1}$ shifts to 1711–1709 cm $^{-1}$ (by 2-4 cm $^{-1}$). Thymine band at 1669 cm $^{-1}$ shifts to 1662–1661 cm $^{-1}$ and adenine band at 1606 cm $^{-1}$ shift to 1601–1600 cm $^{-1}$ in the spectra of Zyloric & uric acid-DNA complexes.

Likewise, cytosine band at 1492 cm $^{-1}$ is observed to 1495–1496 cm $^{-1}$ in the complex spectra. In difference spectra, slight intensity increase is evident for guanine and thymine vibrations whereas intensity decrease is observed for adenine and cytosine vibrations.

These shifting with minor intensity variations are indicative of the intercalative mode of binding of Zyloric & uric acid with DNA. Intercalation occurs through hydrogen bonding with nitrogen base pairs. More shifting is observed for AT base pairs compared to GC pairs. This could explain as Zyloric & uric acid intercalates more between AT base pairs. Prominent shifts have been associated with major sites of interactions while less-prominent with secondary sites.[37,38]Phosphate asymmetric and symmetric stretching bands are assigned at 1221 cm $^{-1}$ and 1086 cm $^{-1}$ respectively. Asymmetric band (1221 cm $^{-1}$) is also a confirmation marker for DNA in B-conformation [39].This band shifts at 1223 cm $^{-1}$ when Zyloric & uric acid is added to the DNA. In difference spectra, positive features are visible around 1233 cm $^{-1}$ and 1101 cm $^{-1}$, showing slight intensity increase of phosphate stretchings.

Sugar-phosphate band at 1055 cm $^{-1}$ shifts to 1054 cm $^{-1}$ upon Zyloric & uric acid-DNA interaction. Similarly the DNA backbone marker band at 970 cm $^{-1}$ shifts downward by 1 cm $^{-1}$ at 969 cm $^{-1}$. Intensity increase is observed for this band in difference spectra. IR band at 894 cm $^{-1}$ is assigned to deoxyribose vibrations. It also shifts to 892 cm $^{-1}$ in the complex spectra.

The IR band at 837 cm $^{-1}$ is also assigned as a marker band for DNA in B-conformation. This band arises due to sugar-phosphodiester chain vibrations and is very sensitive for any subtle change in S-type conformation of the deoxyribose sugar of DNA [40, 41]. Major shifts of 7–8 cm $^{-1}$ are observed for this band. Intensity increment is also accompanied to the shifting. These all spectral features are indicative of glycosidic bond torsion due to AU intercalation between nitrogenous bases.

This intercalation weakens inter-strand hydrogen bonding which results in distortion of the glycosidic bond angle. This distortion hence creates some N-type conformational features in sugar puckering. Shifting in all the mentioned bands at 1713, 1221, 1055, 970, 892 and 837 cm $^{-1}$ could be attributed to some perturbation of DNA conformation upon interaction with Zyloric & uric acid.

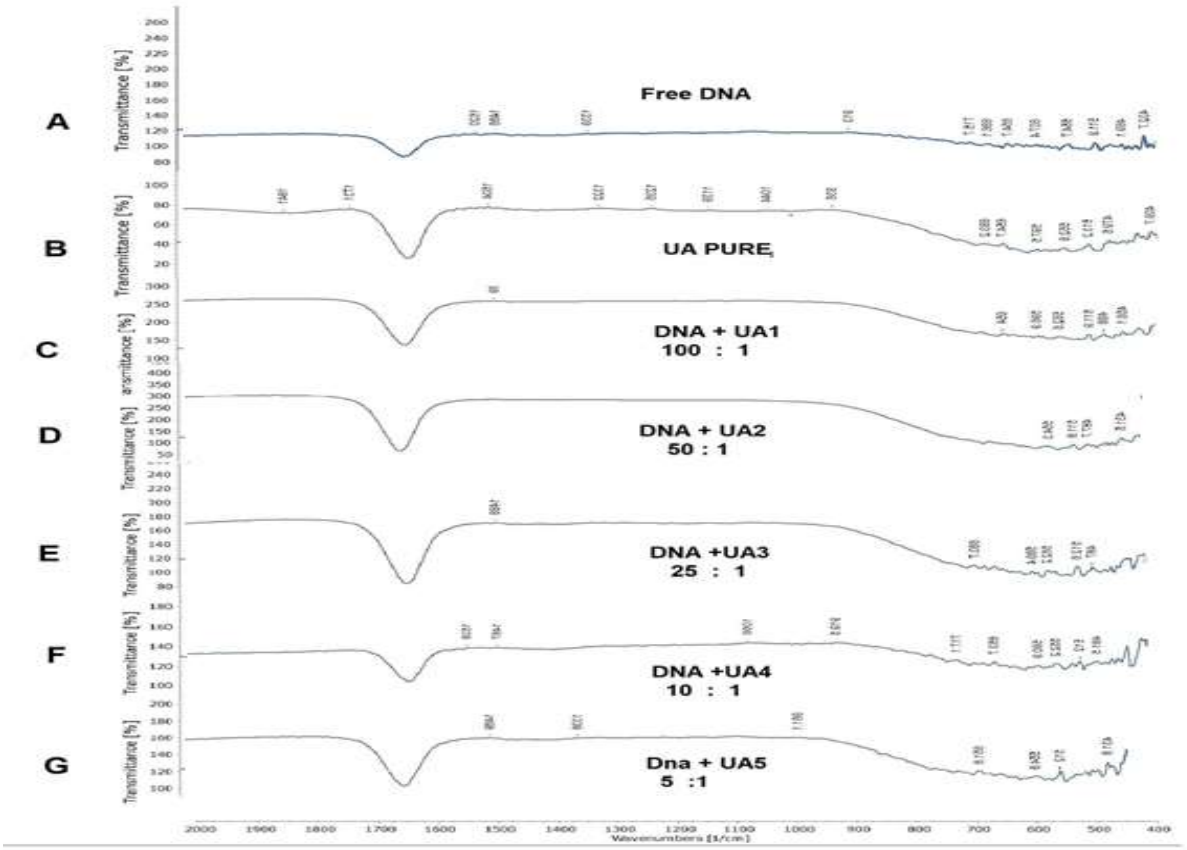

Figure 6: Shows FTIR spectra of free DNA and its complexes with different molar ratios of uric acid

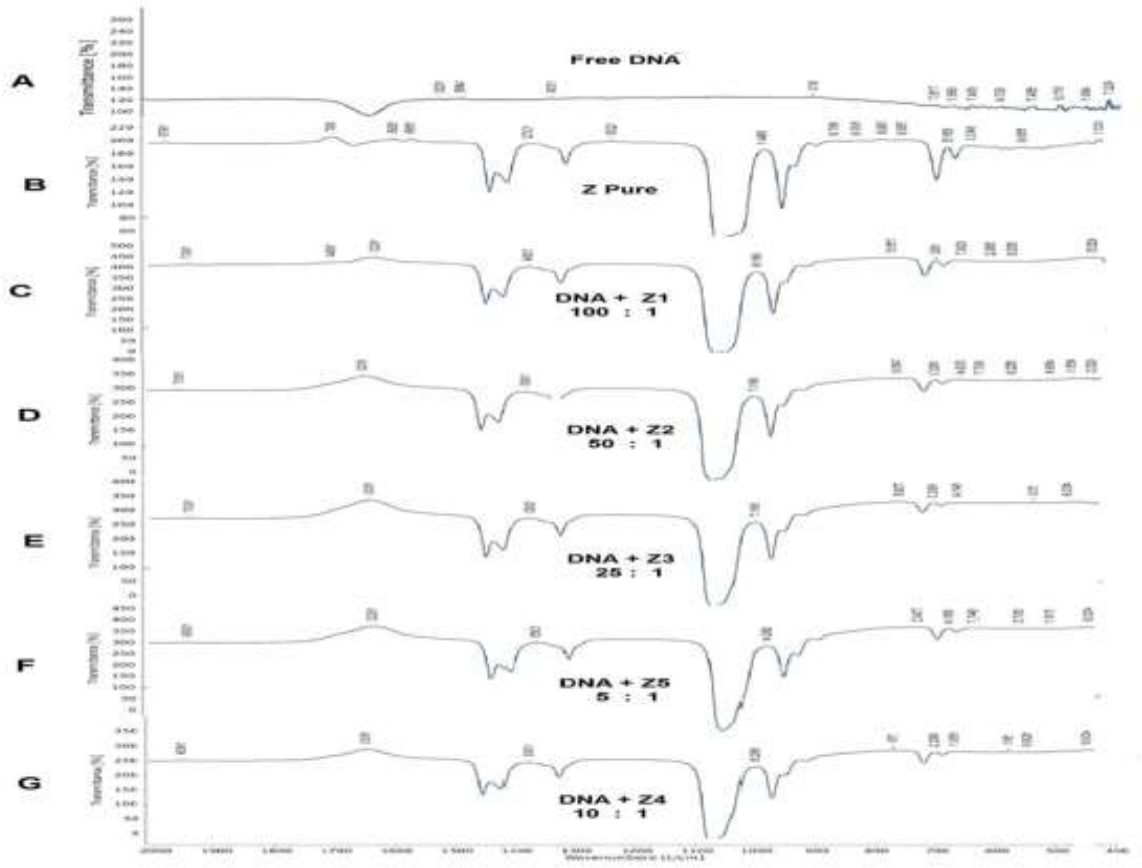

Figure 7: Shows FTIR spectra of free DNA and its complexes with different molar ratios of Zyloric

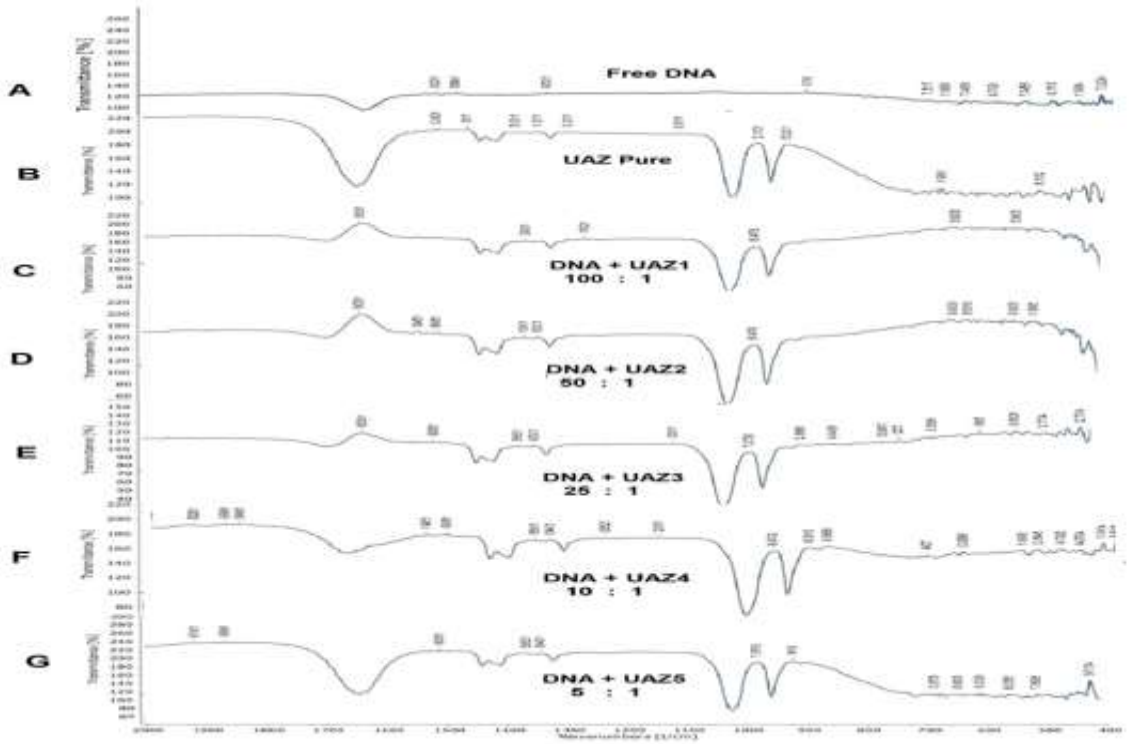

Figure 8: Shows FTIR spectra of free DNA and its complexes with different molar ratios of Zyloric & uric acid

Conclusion

Through our study we find that there is a strong correlation between many variables and Chronic renal failure, and from which we concluded the following, there is a high level of urea, uric acid and creatinine for patients, low DNA concentration in patients, the DNA concentration in whole blood is higher than its concentration in serum, an inverse relationship between DNA concentration and uric acid concentration and there is an overlap of intercalative binding type for uric acid and zyloric with DNA.

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