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Animal Model of Diabetic Nephropathy Associated with Hypertension: Identifying Urinary Tract Infection Biomarkers Protected by Antidiabetic Drugs, Similar to Patients with Uncontrolled Diabetes

Modelo Animal de Nefropatía Diabética Asociada a Hipertensión: Identificación de Biomarcadores de Infección del Tracto Urinario Protegidos por Fármacos Antidiabéticos, Similares a los de Pacientes con Diabetes no Controlada

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SUMMARY: Urinary tract infection (UTI) is common in patients with uncontrolled diabetes mellitus. However, the relationship between diabetic nephropathy associated with hypertension (DNH) and two key UTI markers–leukocyturia and urinary nitrite–has not previously been investigated in animal models, either in the presence or absence of antidiabetic drug treatment. In humans, establishing a direct association between diabetic nephropathy and hypertension is challenging, as ageing independently contributes to arterial stiffening and elevated blood pressure. To overcome this limitation, DNH was induced in age-matched, non-diabetic, and normotensive rats, allowing the effects of diabetes-related hypertension to be examined independently of ageing. Therefore, in addition to the control and untreated diabetic rats, two diabetic groups were treated with metformin (200 mg/kg) and metformin plus sitagliptin (200 mg/kg plus 100 mg/kg sitagliptin), and all animals were culled at week 12 for histopathological, morphological, immunohistochemical, biochemical, and physiological analyses. DNH was confirmed in the diabetic group, as demonstrated by increased kidney tissue levels of nitrosative stress and inflammatory cell infiltration, kidney mesangial expansion and tubular necrosis, renal fibrosis, albuminuria, and hypertension. Additionally, all untreated rats with DNH tested positive for leukocyturia and urinary nitrite in addition to exhibiting proteinuria and ketonuria. In contrast, all control rats showed negative results, and no haematuria was observed in either group. Furthermore, 92 % of rats treated with the antidiabetic drugs demonstrated negative glucosuria, which correlated with a substantial reduction in UTI biomarkers. Notably, metformin plus sitagliptin exhibited the most effective protection. Thus, we induced a rat model of DNH supported by pathological and morphological changes, and identified urinary nitrite and leukocyturia as potential additional biomarkers of DNH, while antidiabetic treatments confer protective effects beyond glycaemic control. Our data reflect patterns observed in uncontrolled diabetes in humans, and suggests that their detection may warrant early clinical investigation and intervention.

KEY WORDS: Diabetic nephropathy; Hypertension; Biomarkers; UTI; Antidiabetic drugs; Animal model.

INTRODUCTION

Diabetic complications such as diabetic nephropathy (DN), which affects the kidneys is the leading cause of end-stage renal failure, and about 30 % to 40 % of diabetic patients develop DN (Gheith *et al.*, 2016). In DN,

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uncontrolled hyperglycemia damages the filtering units of the kidney (the glomeruli), leading to proteinuria, decreased glomerular filtration rate, and eventually, loss of kidney function (Satirapoj & Adler, 2014). A rapid rise in obesity in genetically vulnerable individuals can cause diabetes associated with renal dysfunction, contributing to DN (Shen *et al*., 2010; Bayliss *et al*., 2012). Early signs of DN often include the presence of protein in the urine (microalbuminuria) and may also involve other indicators such as increased blood pressure (DNH) (Van Buren & Toto, 2011). Therefore, regular monitoring of kidney function through urine tests such as microalbuminuria, as well as blood pressure are crucial for early detection and management (Ali *et al*., 2014).

People with diabetes have a higher risk of developing urinary tract infections (UTIs), which can be more severe, result in increased hospitalizations and mortality, and may take longer to treat (Nitzan *et al*., 2015; Confederat *et al*., 2023). In addition, there is a higher chance of recurrent infections; therefore, appropriate antibiotic treatment for symptomatic cases of UTI in diabetic patients is crucial to decrease bacterial resistance (Nitzan *et al*., 2015). This is because hyperglycemia in diabetes weakens the immune system, making it harder for the body to fight off infections (Berbudi *et al*., 2020), and glucosuria (glucose in urine) creates a better environment for bacterial growth in the urine; provides a nutrient source for pathogenic bacteria and increases the adhesion of virulent bacteria to the human bladder epithelial cells (John *et al*., 2021).

People with diabetes, particularly women, may also experience bladder dysfunction or nerve damage (diabetic neuropathy), which can lead to incomplete emptying of the bladder, causing urine stagnation, which further increases the risk of infection (Confederat *et al*., 2023). Additionally, poor glycemic control and longer duration of diabetes are among the risk factors that increase the likelihood of UTIs in diabetic patients (Confederat *et al*., 2023). Therefore, managing blood glucose levels is essential in reducing the risk of UTIs. Early diagnosis, proper antibiotic treatment, and good blood sugar control are key to managing UTIs in diabetic patients.

Leukocyturia and the presence of nitrites in the urine are two important indicators often used to diagnose UTIs (Al Lawati *et al*., 2024). Leukocyturia refers to the presence of leukocytes (white blood cells) in the urine, which indicates an immune response to infection or inflammation in the urinary system. Nitrites, on the other hand, are formed when certain bacteria, such as Escherichia coli convert dietary nitrates into nitrites in the urine via the nitrate reductase enzyme (Nair & Seelam, 2021). Together, leukocyturia and nitrites in a urinalysis provide strong evidence for a UTI and guide the need for further testing or antibiotic treatment.

In diabetic patients, leukocyturia may suggest a UTI, to which they are more prone due to the aforementioned reasons; impaired immune function and glycosuria, which create an ideal environment for bacterial growth. This leads to nitrite production, which can then be detected in a standard urinalysis (Berbudi *et al*., 2020; John *et al*., 2021). In the context of DN, recurrent or untreated UTIs can exacerbate kidney damage, accelerating the decline in renal function (Nitzan *et al*., 2015). Therefore, early detection and management of infections are crucial in patients with diabetic kidney disease. However, little is known about the association between DNH and the presence of white blood cells and nitrites in urine in animal models, in the presence and absence of antidiabetic drugs. Thus, the aim of this study was to thoroughly investigate these working hypotheses.

MATERIAL AND METHOD

Animals

The study was conducted in accordance with the Declaration of Helsinki and approved by the Research Ethics Committee at King Khalid University (ECM#2023-3213; dated 15 November 2023). Wistar male rats weighing 170-200 gram per rat were used in this study and were given free access to water and food and kept in a clean animal house with a 12-hour light/dark cycle.

Experimental design.

After an adaptation period, 24 rats were indiscriminately distributed into four groups. Control group (Control): non-treated, non-diabetic rats fed for 12 weeks with standard laboratory chow. Diabetic type 2 group (Diabetes): a combination of a high carbohydrate and fat diet (HCFD) and a single injection of streptozotocin (STZ) was used to induce diabetes mellitus as previously described (El-Bidawy *et al*., 2021). These rats were kept on a HCFD until the end of week 12. The diabetic treated groups; with metformin (200 mg/kg) and metformin plus sitagliptin (200 mg/kg plus 100 mg/kg sitagliptin): animals were given these medications from day one and were fed HCFD for two weeks before diabetes was induced. For another 10 weeks, the rats were given these drugs and were fed a HCFD. Then, blood samples were collected under anesthesia (sodium thiopentone at 40 mg/kg body weight) and rats were euthanized by cervical dislocation and kidney tissue samples were harvested. One week after STZ injection, diabetes was confirmed in the model group by measuring fasting blood glucose levels (> 200 mg/dl).

Determination of glucose, HbA1c, AGEs, hs-CRP, TNF-α, IL-6, urea, creatinine, urine white and red blood cells, nitrite, albumin, ketones, pH, specific gravity, and glucosuria

Blood glucose was measured using a Randox reagent kit (Sigma-Aldrich, UK). Colorimetric methods were used to test blood urea and creatinine, as directed by the manufacturer (BioAssay System, USA). An enzyme-linked immunosorbent assay (ELISA) kits were used to measure glycated hemoglobin (HbA1c) (Crystal Chem Inc, Elk Grove Village, IL, USA), advanced glycation end products (AGEs) (Ray Biotech, USA), high sensitivity C-reactive protein (hs-CRP) (ASSAYPRO, St Charles, MO, USA), tumor necrosis factor-alpha (TNF-α and interleukin-6 (IL-6) obtained from Biotang Inc, Lexington, MA, USA. Urine collection for 24 hours in containers was done using metabolic cages. Microalbuminuria ELISA was used to measure urinary albumin levels (DiaComp, USA). Urine dipsticks were used to test for white and red blood cells, nitrite, albumin, ketones, pH, specific gravity, and glucosuria (Williams Medical Supplies, Gwent, UK).

Assessment of kidney pathology and immunostaining.

Harvested kidney specimens were fixed in 10 % formal saline before dehydration with ascending grades of alcohol and embedded in paraffin as previously reported (Al-Ani *et al*., 2021). Paraffin blocks were sectioned into 5 mm thick slices and stained with Periodic acid Schiff (PAS). Sirius red staining of kidney tissue sections was used to assess collagen deposition in kidney. Embedded kidney slices were dewaxed in xylene and rehydrated in water via a series of graded alcohols. After an overnight incubation with 0.1 percent Sirius red (Sigma-Aldrich, UK), the slides were dipped in 0.01 M hydrochloric acid and dehydrated with progressive ethanol doses without water.

Tissue slices were also immunostained with anti-iNOS and anti-CD45 antibodies (Abcam, Cambridge, UK) overnight in cold room at dilution 1:100 and processed as previously reported (Dawood *et al*., 2022). Morphometry of the iNOS and CD45 immunostaining area percent, the area percent collagen deposition in Sirius Red stained sections and area of mesengial expansion were estimated in 10 non-overlapping fields for each group using a "Leica Qwin 500 C" image analyzer (Cambridge, UK). The means and standard deviations (SD) of quantitative data were calculated and compared using analysis of variance (ANOVA) and post-hoc analysis (Tukey test). P-values less than 0.05 were deemed statistically significant. The calculations were done using version 19 of the statistical package for social science (SPSS) software.

Determination of systolic, diastolic, and mean arterial blood pressure. These parameters were measured in conscious rats as we previously reported (Dallak *et al*., 2019) using the tail-cuff technique.

Statistical analysis

Data were expressed as means ± SD. SPSS version 10.0 was used to analyze the data (SPSS, Inc., Chicago, Ill., USA). Tukey's post hoc test was used after performing a one-way ANOVA. For the purpose of detecting a possible relevance between two separate values, a Pearson correlation statistical analysis was used. A probability value of ≤ 0.05 was considered significant.

RESULTS

Induction of diabetic nephropathy associated with hypertension (DNH) in rats.

We first modelled the disease in rats in order to correlate kidney tissue markers of inflammation and nitrosative stress as well as kidney injury, fibrosis, and hypertension (DNH) with urinary leukocytes and nitrite. Induction of type 2 diabetes mellitus for 10 weeks caused a sharp increase in biomarkers of kidney injury and hypertension with evidence of pathological and morphological changes in the prepared kidney tissue sections and renal arteries (Figs. 1 and 2). In comparison with control group, diabetic rats showed a significant ($p \leq 0.0006$) increase in biomarkers of diabetes; blood glucose (Fig. 1A), glycated haemoglobin (HbA1c; Fig. 1B), and massive accumulation of advanced glycation end products (AGEs) in the renal artery tissue (Fig. 1C). Glycaemia also caused a sharp increase in kidney tissue levels of nitrosative stress (iNOS protein expression) (Figs. 1E & 1F) and infiltration of inflammatory leukocytes (CD45 +ve cells) (Figs. 1H and 1I) compared to a negative tissue expression in control rats (Figs. 1D & 1G), which substantially increased the inflammatory biomarkers; hs-CRP (Fig. 1J), TNF-α (Fig. 1K), and IL-6 (Fig. 1L). All these deleterious effects of hyperglycaemia were significantly inhibited by the antidiabetic drug metformin (data not shown).

We further assessed the impact of upregulation of the kidney iNOS/CD45/inflammation axis by diabetes on kidney structure and function. Data obtained from diabetic untreated rats displayed a substantial increase in urine albumin (Fig. 2A), blood urea (Fig. 2B), and creatinine (Fig. 2C), compared with normal values for the control. Additionally, prepared kidney sections were stained with a special histological stain; periodic acid Schiff (PAS) (Figs. 2D and 2E); and Sirius red (Figs. 2G and 2H). Strong PAS

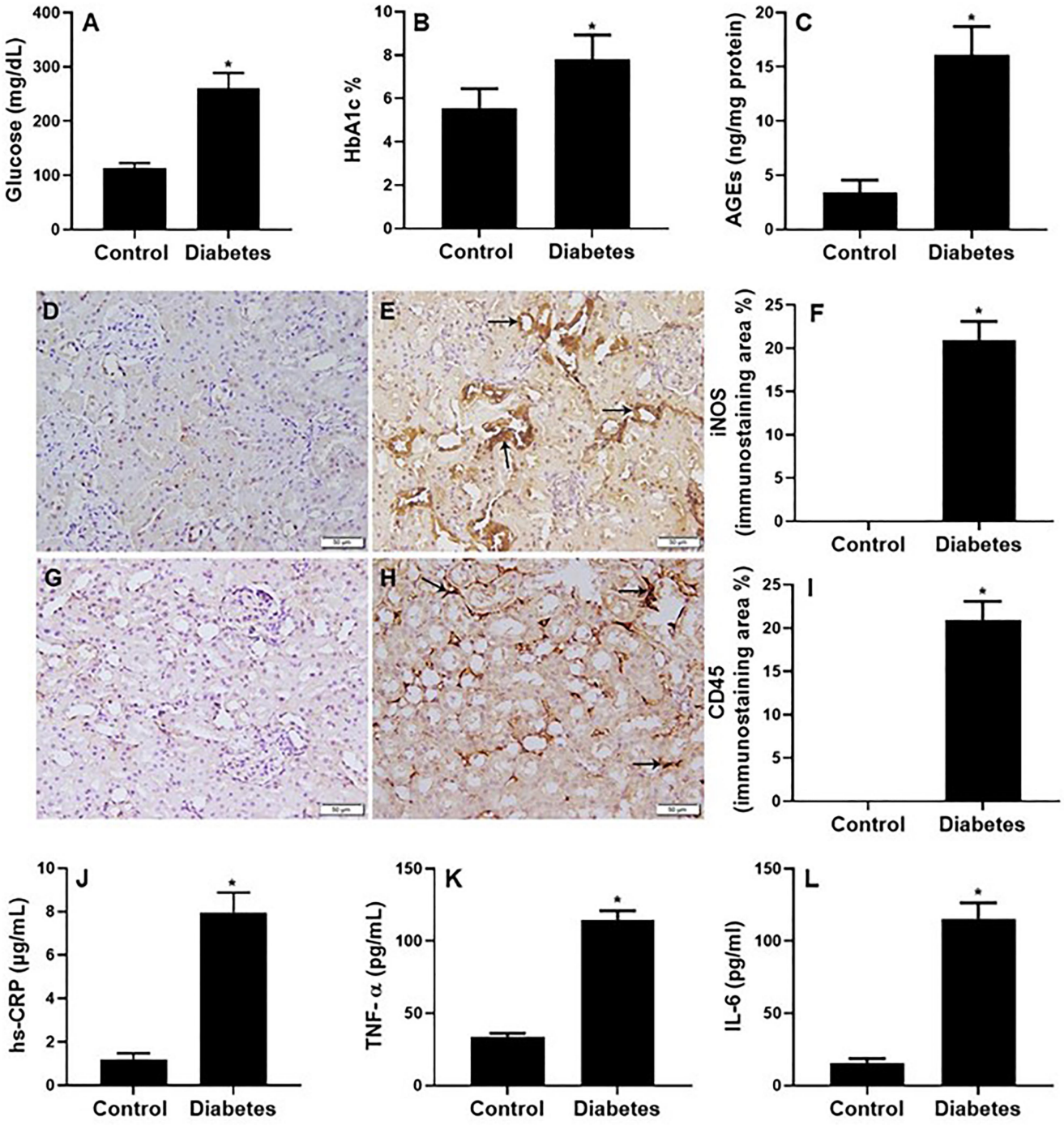

Fig. 1. Induction of kidney iNOS/CD45/inflammation axis by diabetes. Blood levels of glucose (A) and HbA1c (B), as well as renal artery AGEs (C) were measured 10 weeks post diabetic induction in the control and diabetic groups. Representative immunohistochemistry images (200x) of iNOS (D and E) and CD45 (G and H) of kidney sections from the control rats (D and G) and diabetic rats (E and H) are shown. Bar graphs in (F and I) denote a quantitative analysis of iNOS immunostained area % (F) and CD45 immunostained area % (I) in kidney sections from these groups. (J-L) Inflammatory biomarkers hs-CRP (J), TNF-a (K), and IL-6 (L) were measured at the end of the experiment in all rats. *$p < 0.0006$ versus control. HbA1c: glycated haemoglobin; AGEs: advanced glycation end products; iNOS: inducible nitric oxide synthase; CD45: common leukocyte antigen; hs-CRP: high sensitivity C-reactive protein; TNF-α: tumor necrosis factor-alpha; IL-6: interleukin-6.

positive reaction in the model group (Fig. 2E) indicates that diabetes triggered expansion in the mesangial regions (M), thickening of capsular and tubular basement membranes (wavy arrows), with the loss of brush border of some tubular epithelium (asterisk) compared with normal kidney architecture in the control group (Fig. 2D). Quantitative analysis of area mesangial expansion displayed a significant ($p < 0.0001$) increase caused by diabetes compared to control (Fig. 2F). Diabetes also (i) substantially induced kidney fibrosis (Figs. 2H and 2I) as demonstrated by perivascular collagen deposition and in the renal interstitium surrounding renal corpuscles and the convoluted tubules (arrowheads) compared with fine collagen staining in the control (Figs. 2G and 2I); and (ii) substantially induced systolic blood pressure (Fig. 2J), diastolic blood pressure (Fig. 2K), and mean arterial blood pressure (Fig. 2L) since diabetic nephropathy is strongly associated with high blood pressure (Van Buren & Toto, 2011), and hypertension can cause more kidney damage (Giunti *et al*., 2006). As expected, diabetes significantly ($p < 0.0001$) reduced body weight in gram (270.0 ± 5.25 for control rats; versus 163.3 ± 7.52 for diabetic rats; versus 305.83 ± 31.05 for diabetic rats treated with metformin). Metformin also protected against diabetes-induced all the above assessed parameters (data not shown).

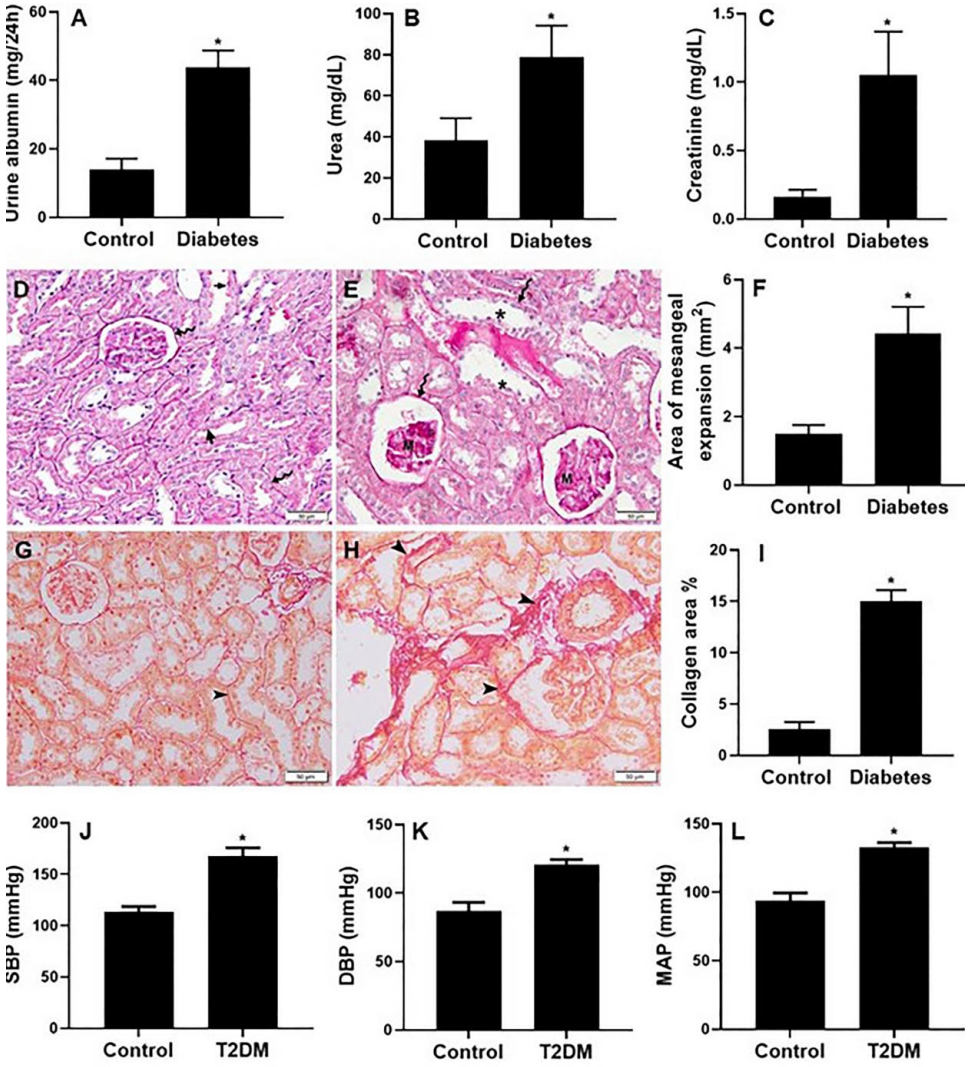

Fig. 2. Diabetes induces chronic kidney disease and hypertension. Levels of urine albumin were assessed at the end of the experiment (A), as well as blood urea (B) and creatinine (C). PAS-stained images (200x) of kidney sections from the control (D) and diabetic (E) groups are shown. Sirus red-stained images (200x) of kidney sections from the control (G) and diabetic (H) groups are illustrated. Bar graphs in (F) and (I) represent a quantitative analysis of mesangial expansion and collagen deposition, respectively. (J-L) SBP (J), DBP (K), and MAP (L) values were measured at the end of the experiment in these animal groups. *$p < 0.0001$ versus control. PAS: periodic acid Schiff; SBP: systolic blood pressure; DBP: diastolic blood pressure; MAP: mean arterial blood pressure.

Induction of diabetic nephropathy associated with hypertension (DNH) is linked to the presence of leukocyturia and urine nitrite

Diabetes is a recognised risk factor for the development of urinary tract infections (UTI) (Ahmed *et al*., 2023). Therefore, in view of the results described above showing dysregulation of certain cell signalling pathways that led to the development of DNH in our animal model, a 24-hour urine collection from all rats at the end of the experiment was performed to assess two key UTI indicators; urine white blood cells and nitrite. Table I shows urine data collected from the control rats that demonstrated negative results for white and red blood cells, nitrite, ketone bodies, and glucose in all samples. In contrast, urine data collected from the untreated diabetic rats at week 12 (Table II) showed positive results for white blood cells and nitrite, as well as the presence of proteinuria, ketone bodies, and glucose in all samples. Interestingly, haematuria was negative in all samples.

Diabetic drugs inhibit leukocyturia and urine nitrite induced by diabetes

In light of our findings described above that demonstrated a strong association between DNH and positive urine leukocytes and nitrite, we tested the protective effects of the antidiabetic drugs. It involved two groups of rats treated with oral antiglycemic agents (metformin and a combination of metformin and sitagliptin) both before and after diabetic induction. As shown in Tables III and IV, oral hypoglycemic drugs inhibited glucosuria, which was associated with the inhibition of leukocyturia and nitrite in urine as well as proteinuria and ketonuria.

Table I. Urine analysis for the control group of rats at week 12. Urine levels of white blood cells (WBCs), nitrite, protein, blood, ketone, glucose, pH, and specific gravity were estimated at the end of the experiment.

Control group	**WBCs** (WBC/ml)	**Nitrite**	**Protein** (mg/dL)	**pH**	**Blood** (RBC/ml)	**Specific gravity**	**Ketone** (mmol/L)	**Glucose** (mmol/L)
Rat #1	Negative	Negative	Trace	7.5	Negative	1005	Negative	Negative
Rat #2	Negative	Negative	Trace	7	Negative	1005	Negative	Negative
Rat #3	Negative	Negative	Trace	7	Negative	1005	Negative	Negative
Rat #4	Negative	Negative	Trace	7	Negative	1005	Negative	Negative
Rat #5	Negative	Negative	Trace	7.5	Negative	1010	Negative	Negative
Rat #6	Negative	Negative	Trace	7	Negative	1005	Negative	Negative

Table II. Urine analysis for the model group of rats at week 12. Urine levels of white blood cells (WBCs), nitrite, protein, blood, ketone, glucose, pH, and specific gravity were estimated at the end of the experiment.

Diabetic group	**WBCs** (WBC/ml)	**Nitrite**	**Protein** (mg/dL)	**pH**	**Blood** (RBC/ml)	**Specific gravity**	**Ketone** (mmol/L)	**Glucose** (mmol/L)
Rat #1	70	Positive	0.3	5	Negative	1030	3.9	111
Rat #2	70	Positive	0.3	5	Negative	1030	3.9	111
Rat #3	70	Positive	0.3	5	Negative	1030	3.9	111
Rat #4	70	Positive	0.3	5	Negative	1030	8	111
Rat #5	70	Positive	1	5	Negative	1030	8	111
Rat #6	70	Positive	0.3	5	Negative	1030	3.9	111

Table III. Urine analysis for the metformin-treated group of rats at week 12. Urine levels of white blood cells (WBCs), nitrite, protein, blood, ketone, glucose, pH, and specific gravity were estimated at the end of the experiment.

Treated group	**WBCs** (WBC/ml)	**Nitrite**	**Protein** (mg/dL)	**pH**	**Blood** (RBC/ml)	**Specific gravity**	**Ketone** (mmol/L)	**Glucose** (mmol/L)
Rat #1	Trace	Negative	Negative	6	Negative	1020	Negative	Negative
Rat #2	Trace	Trace	Negative	6	Negative	1030	0.5	Negative
Rat #3	Negative	Negative	Trace	5	Negative	1025	3.9	5.5
Rat #4	Trace	Negative	Trace	6	Negative	1020	1.5	Negative
Rat #5	Trace	Negative	Trace	6	Negative	1015	Negative	Negative
Rat #6	Negative	Negative	Trace	6	Negative	1015	Negative	Negative

Table IV. Urine analysis for the metformin plus sitagliptin-treated group of rats at week 12. Urine levels of white blood cells (WBCs), nitrite, protein, blood, ketone, glucose, pH, and specific gravity were estimated at the end of the experiment.

Treated group	WBCs (WBC/ml)	Nitrite	Protein (mg/dL)	pH	Blood (RBC/ml)	Specific gravity	Ketone (mmol/L)	Glucose (mmol/L)
Rat #1	Trace	Negative	Negative	6	Negative	1030	Negative	Negative
Rat #2	Trace	Negative	Negative	6	Negative	1030	Negative	Negative
Rat #3	Negative	Negative	Negative	6	Negative	1030	Negative	Negative
Rat #4	Trace	Negative	Negative	6	Negative	1030	Negative	Negative
Rat #5	Negative	Negative	Trace	6	Negative	1030	Negative	Negative
Rat #6	Trace	Negative	Negative	5	Negative	1025	0.5	Negative

DISCUSSION

These studies induced in rats an animal model of diabetic nephropathy associated with hypertension (DNH) using age-matched, non-diabetic, and normotensive rats. This model demonstrated all the characteristics of chronic kidney disease secondary to diabetes (identified 17 different pathological and morphological parameters) (Figs. 1 and 2), which enabled us to thoroughly investigate the link between DNH and two key UTI indicators; urine white blood cells and nitrite with and without antidiabetic drug incorporation. Therefore, here we have shown that diabetic induction caused after 10 weeks positive urine results for white blood cells and nitrite in 100 % of the rats with DNH (Table II) compared to 0 % in the control non-diabetic, non-treated rats (Table I). No haematuria was observed in either group. In addition, we have been able to show the disappearance of positive urine nitrite and white blood cells in 100% of animals treated with the antidiabetic drugs (Tables III and IV). This demonstrates an important association between leukocyturia and urinary nitrite with uncontrolled diabetes in a rat model of DNH as well as highlights the renoprotective effects of antidiabetic drugs (Fig. 3).

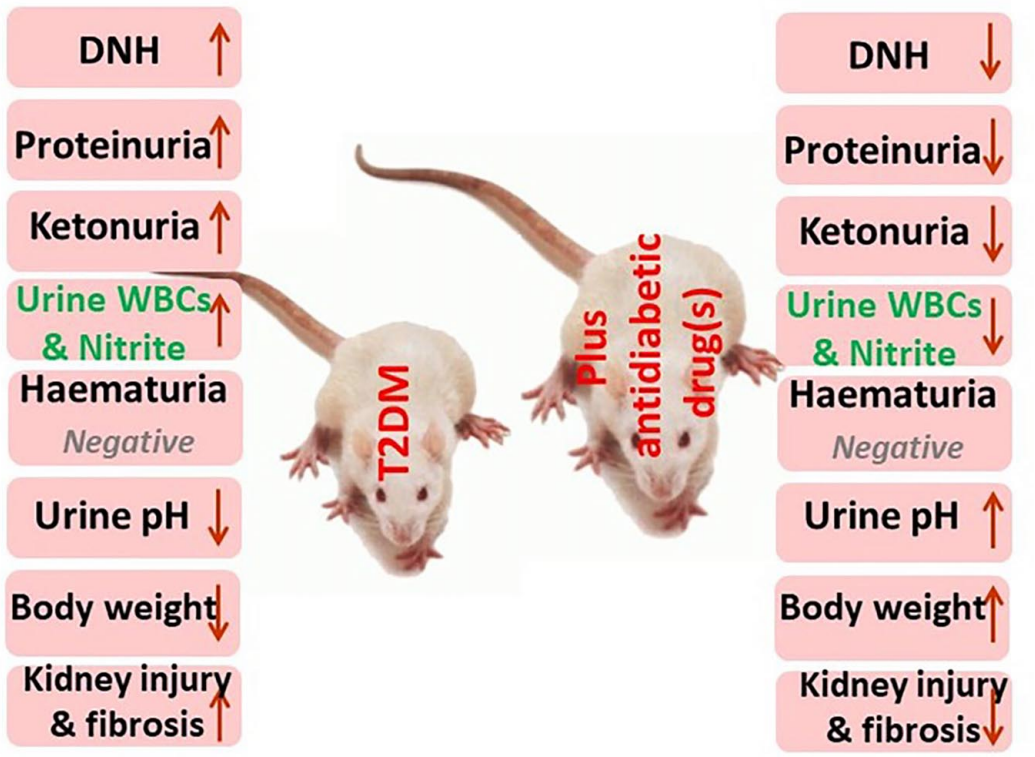

Fig. 3. Proposed model for the association of urine white blood cells and nitrite with DNH which appears to be inhibited by antidiabetic drugs. WBCs: white blood cells; pH: potential of hydrogen; DNH: diabetic nephropathy associated with hypertension.

The lower urinary tract – usually the bladder – of the urinary system is the part most commonly affected by bacterial infections (commonly known as lower UTI), which keeps coming back with certain types of pathogenic bacteria (Dason *et al*., 2011; Zhou *et al*., 2023), and diabetes is known to increase the risk of getting a UTI, up to 10 times higher risk (Nitzan *et al*., 2015). Indeed, (i) the prevalence of UTI in diabetic patients was estimated to be between 20 to 35% (Al-Rubeaan *et al*., 2013; Sewify *et al*., 2016); (ii) patients with type 2 diabetes mellitus (T2DM) experience a UTI and recurrent UTIs more than subjects without T2DM (Fu *et al*., 2014); (iii) a study reported that 53% of diabetic dogs had leukocyturia (Nelson *et al*., 2023); and (iv) uncontrolled diabetes represented the majority of diabetic patients with UTI compared to controlled diabetes (Sewify *et al*., 2016). These reports corroborate our findings that correlate with these parameters. Additionally, hypertension and nephropathy (microalbuminuria) have been reported as risk factors association with UTI in diabetic patients (Al-Rubeaan *et al*., 2013). However, the authors noted difficulty in establishing a link between hypertension and UTI in diabetes because 32 % of the enrolled diabetic patients in the study were over 60 years old, and the risk of hypertension increases significantly with age. Moreover, the study did not include non-diabetic control patients for comparison. Consequently, it is often difficult to determine whether hypertension is a direct consequence of diabetes or an age-related physiological change. In contrast, in our animal model of DNH, we included age-matched, non-diabetic, normotensive control rats, which is a crucial control group in diabetes/hypertension research to compare against the disease model. These controls help rule out confounding variables, making our findings on disease progression and treatment efficacy more reliable and translatable to humans.

Collectively, our histological and morphological, immunohistochemical, biochemical, and physiological data support the conclusion that we have generated an animal model of DNH that was used to demonstrate a strong association between uncontrolled diabetes and leukocyturia and urinary nitrites, showing a comparable predictive value to proteinuria and ketonuria, both of which showed substantial inhibition by antidiabetic drugs. Therefore, this study highlights these two biomarkers as additional indicators for DNH. Furthermore, quantitative validation and microbiological confirmation of UTI are recommended for future studies to further support our findings in this animal model. This is despite the fact that (i) a positive predictive value (PPV) of 85 % has been reported when combining the leukocyte esterase (LE) and nitrite tests using a urine dipstick for UTI (Bellazreg *et al*., 2019), and (ii) our approach is non-invasive and very inexpensive.

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RESUMEN: La infección del tracto urinario (ITU) es frecuente en pacientes con diabetes mellitus no controlada. Sin embargo, la relación entre la nefropatía diabética asociada a la hipertensión (NDH) y dos marcadores clave de ITU (leucocituria y nitrito urinario) no se había investigado previamente en modelos animales, ni en presencia ni en ausencia de tratamiento con fármacos antidiabéticos. En humanos, establecer una asociación directa entre la nefropatía diabética y la hipertensión es un desafío, ya que el envejecimiento contribuye de forma independiente a la rigidez arterial y a la elevación de la presión arterial. Para superar esta limitación, se indujo NDH en ratas normotensas, no diabéticas y de la misma edad, lo que permitió examinar los efectos de la hipertensión relacionada con la diabetes independientemente del envejecimiento. Por lo tanto, además de las ratas control y las diabéticas no tratadas, dos grupos diabéticos fueron tratados con metformina (200 mg/kg) y metformina más sitagliptina (200 mg/kg más 100 mg/kg de sitagliptina), y todos los animales fueron sacrificados en la semana 12 para análisis histopatológicos, morfológicos, inmunohistoquímicos, bioquímicos y fisiológicos. Se confirmó la DNH en el grupo diabético, como lo demuestran los niveles elevados de estrés nitrosativo e infiltración de células inflamatorias en el tejido renal, la expansión mesangial renal y la necrosis tubular, la fibrosis renal, la albuminuria y la hipertensión. Además, todas las ratas no tratadas con DNH dieron positivo para leucocituria y nitrito urinario, además de presentar proteinuria y cetonuria. En contraste, todas las ratas control mostraron resultados negativos y no se observó hematuria en ninguno de los grupos. Asimismo, el 92 % de las ratas tratadas con fármacos antidiabéticos mostraron glucosuria negativa, lo que se correlacionó con una reducción sustancial de los biomarcadores de ITU. Cabe destacar que la combinación de metformina y sitagliptina mostró la protección más eficaz. Por lo tanto, indujimos un modelo de rata con DNH respaldado por cambios patológicos y morfológicos, e identificamos el nitrito urinario y la leucocituria como posibles biomarcadores adicionales de DNH, mientras que los tratamientos antidiabéticos confieren efectos protectores más allá del control glucémico. Nuestros datos reflejan patrones observados en la diabetes no controlada en humanos y sugieren que su detección podría justificar una investigación e intervención clínica tempranas.

PALABRAS CLAVE: Nefropatía diabética; Hipertensión; Biomarcadores; Infección del tracto urinario; Fármacos antidiabéticos; Modelo animal.

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