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ORIGINAL RESEARCH **PERIOPERATIVE MEDICINE**

Serum S100A8/A9 and RNA-binding protein, tristetraprolin as prognostic markers of renal damage

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ABSTRACT

Background & objective: The kidneys are vital and complex organs responsible for maintaining normal body functions. Kidney disease or a loss of kidney function and, in severe cases, complete kidney failure disrupt whole of the body systems. Tristetraprolin (TTP ZFP36) is an RNA-binding proteins (RBP), that regulates cytokine mRNAs by specific binding of its two conserved ZF domains to adenylate-uridylate-rich elements (AREs) at the untranslated region (UTR), leading to degradation of the RNA. Recently, it was suggested that S100 A8/A9 and TTP were involved in pathogenesis of renal diseases. However, the protective roles of S100 A8/A9 and TTP in renal disease is still unclear. The primary objective of this study was to explore the involvement of S100 A8/A9 and TTP in the inflammatory response among different groups of kidney diseases. These factors may hold therapeutic value in the treatment of these diseases by targeting and modulating the inflammatory response.

Methodology: The study was performed between October 2022 to April 2023. We included 150 subjects, including 30 patients with chronic kidney disease (CKD), (Female = 16, Male = 14) with mean age (58.33 ± 18.33 y), 30 patients with acute kidney injury (AKI), (Female = 15, Male = 15), with mean age (54.48 ± 18.10 y), 30 patients with diabetic nephropathy (DN), (Female = 18, Male = 12), with mean age (60.84 ± 12.38 y), 30 patients with nephrotic syndrome (NS), (Female = 13, Male = 17), with mean age (52.54 ± 14.02 y), and 30 healthy persons as a control group. NLR analysis was performed directly using the hematology analyzer CBC (Sysmex, Japan) technique, serum S100 A8/A9 were measured by ELISA, and TTP gene expression was measured by Quantitative Real Time-Polymerase Chain Reaction (RT-qPCR).

Results: In comparison to the healthy control group, all patient groups exhibited a significant increase in the NLR ($P < 0.05$). Furthermore, the findings clearly demonstrated elevated levels of S100 A8/A9 in all patient groups when compared to the control group ($P \leq 0.05$). The results also demonstrated a significant down regulation in the expression of TTP in DN and nephrotic syndrome (NS) groups compared to the control ($P \leq 0.01$). AKI showed highly significant increase in TTP expression in comparison to control and other patient groups ($P \leq 0.01$). Non-significant decrease in TTP gene expression was indicated in CKD compared to healthy group ($P \geq 0.05$).

Conclusions: The activation of Ca^{2+}-binding protein S100A8/A9 is involved in the renal damage; thus, targeting it could have a therapeutic value in the treatment of kidney diseases. Tristetraprolin is contributed to the regulation of inflammatory events in kidney diseases especially in AKI group due to a rapid and robust inflammatory response that contributes to the pathogenesis of this disease.

Abbreviations: AKI- acute kidney injury ARE- Adenylate-Uridylate-Rich Element; DN- diabetic nephropathy; NS- Nephrotic Syndrome; NLR- Neutrophil-to-Lymphocyte Ratio; RT-qPCR= Quantitative Real Time-Polymerase Chain Reaction; RBP- RNA-Binding Proteins; TTP- Tristetraprolin; UTR- Untranslated Region;

Key words: Kidney diseases; S100 A8/A9, Anti-inflammatory RNA binding protein; Tristetraprolin; Neutrophil/Lymphocyte Ration

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1. INTRODUCTION

The kidneys play a crucial role in maintaining the body's normal functions by overseeing fluid and electrolyte balance as well as coordinating the activities of various organ systems. Within the kidney, the nephron stands as the fundamental operational unit, comprising the glomerulus and renal tubules. Together, these components form an intricate and essential system.[1] Renal disease refers to any damage or illness affecting the kidneys, which often leads to a loss of kidney function and, in severe cases, complete kidney failure. Chronic kidney disease (CKD), acute kidney injury (AKI), diabetic nephropathy (DN), and nephrotic syndrome (NS) are common renal diseases characterized by: persistent kidney abnormalities, sudden decline in kidney function within seven days,[2,3] angiopathy of the capillaries in poorly managed diabetes,[4] and specific symptoms including proteinuria, hypoalbuminemia, high blood lipids, and significant swelling,[5] respectively.

S100s are a cluster of proteins that have the ability to bind calcium. They belong to the superfamily of calcium-binding EF-hand proteins. Their name originates from their characteristic solubility in a solution that is 100% saturated with ammonium sulfate at a neutral pH.[6] S100 family of proteins contains 25 known members that share a high degree in their amino acid sequence, structural similarity and have a similar molecular mass of 10–12 KDa. From the 25 human S100 genes, 19 (group A S100 proteins) are situated on chromosome 1q21. Other members are related to various regions.[7] The EF-hand being a helix-loop-helix structural domain or motif.[8] These proteins possess amino acid residues with charges, which contribute to their attraction towards divalent ions like Ca^{++}, Zn^{++}, and Cu^{++}. The EF-hand sites have a specific affinity for binding calcium ions, while separate sites are responsible for binding zinc and copper ions.[9,10] S100A8 and S100A9, members of the S100 family of calcium-binding proteins, form a heterodimer called calprotectin, which is the most abundant form of these proteins in human serum. Calprotectin, a 24-kDa heterodimer, consists of two monomers: S100A8 (10,835 kDa) and S100A9 (13,242 kDa). Both S100A8 and S100A9 have the ability to bind calcium.[11] S100A8/A9 proteins are released by phagocytes, particularly at sites of inflammation, such as neutrophils, monocytes, and early differentiated macrophages. However, resident tissue macrophages do not secrete these proteins. S100A8/A9 are commonly known as damage-associated molecular patterns or alarmins, and they play crucial roles in modulating inflammatory responses and diseases associated with inflammation.[12] S100A8 is the active component of calprotectin, and S100A9 regulates its activity. These proteins are released by phagocytes at sites of inflammation but not by resident tissue macrophages.

Pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α) and Interleukin-1β (IL-1β), increase the production of calprotectin. Furthermore, lipopolysaccharide (LPS) and interferon-γ (INF-γ) induce the translocation of S100A8 and S100A9 from the cytoplasm to the cell surface.[13] Following their activation and release, the primary mode of action for S100A8/A9 involves interaction with three receptors: pattern recognition receptors (PRRs) such as Toll-like receptor 4 (TLR4), receptor of advanced glycation end-products (RAGE), and the extracellular matrix metalloproteinase inducer (EMMPRIN), also known as CD147 and M6 in human.[14] S100A8/A9 proteins have been associated with the regulation of numerous intracellular and extracellular functions. They play a crucial role in the advancement of inflammation by promoting the migration of neutrophils to inflammatory sites. Additionally, S100A8/A9 induces the release of cytokines from monocytes/macrophages and enhances the activity of nicotinamide adenine dinucleotide phosphate hydrogen (NADPH) oxidase, leading to the production of superoxide (O^{-2}). Furthermore, S100A8/A9 serve as highly sensitive markers for acute inflammation.[15] S100A8/A9 acts as a cytoplasmic Ca^{++} sensor that links Ca^{++} influx to phagosomal reactive oxygen species (ROS) production.[16] Following cellular damage, stress, or activation of immune cells, such as neutrophils and macrophages, S100A8/S100A9 proteins are released into the extracellular space, where they play a crucial role in regulating various immune and inflammatory processes.[17] One notable example is their interaction with TLR4, which triggers a signaling cascade involving nuclear factor κ β (NF-κ β) activation, subsequently modulating inflammation, cell proliferation, differentiation, and tumor development.[18] The ability of S100A8/A9 to activate TLR4 links them to innate immunity.[19] S100A8/S100A9 usually exist as

heterodimeric complexes that form heterotetramers in the presence of calcium. S100A8/S100A9 released by activated phagocytes is only temporarily active in the local extracellular microenvironment due to a mechanism of auto-inhibition that is induced by calcium-dependent tetramerization of S100A8/S100A9. By preventing their interaction with TLR4, the tetramers help control and modulate the functions and effects of S100A8/S100A9 in various biological processes.[20,21] In healthy individuals, the levels of circulating S100A8 and S100A9 usually range from 0.1 to 0.6 mg/L. However, in inflammatory conditions, their levels can increase up to 20 times higher than normal.[10]

RNA-binding proteins (RBPs) are a group of proteins that have the ability to bind to ribonucleic acid (RNA) molecules. They are commonly found in both the cytoplasm and nucleus of cells and play a crucial role in the formation of ribonucleoproteins (RNPs).[22] RBPs form a large class, consisting of over 2000 proteins, which interact with RNA and regulate various aspects of RNA metabolism.[23] Remarkably, RBPs account for approximately 7.5% of protein-coding genes.[24] RBPs play essential roles in various cellular processes such as cell transport, development, localization, differentiation, and metabolism. They are also pivotal in post-transcriptional regulation, influencing transcript formation, function, and contributing to cellular homeostasis.[25] Tristetraprolin (TTP) is the prototype member of the TIS11, ZFP36, family of RNA-binding proteins, which includes three members in humans. The ZFP36 gene is responsible for encoding a zinc finger protein, approximately 36 kDa in size, which contains three repeats of the PPPP-motif. In humans, the ZFP36 gene is located on chromosome 19q13.1 and consists of two exons and one intron.[26] TTP is an RNA-binding protein that regulates cytokine mRNAs by binding specifically to adenylate-uridylate-rich elements (AREs) at the 3′-untranslated region (UTR) of target mRNAs through its two conserved ZF domains (CysX8CysX5CysX3His).[27] This binding leads to degradation of the RNA and recruitment of the CCR4-NOT decapping and deadenylation complex, resulting in destabilization and removal of the mRNA from the cell. TTP targets a wide variety of mRNAs that encode cytokines and other immune-related factors, including CCL2, IL-6, IL-10, TNF-α, and many others.[28] The activity of TTP is regulated by phosphorylation by p38-MAPK-activated protein kinase 2 (MK2), which inhibits the mRNA destabilizing activity of TTP. MK2 phosphorylates TTP at two serine residues, which reduces its affinity for ARE-RNA sequences and inhibits its RNA-binding activity.[29] When TTP is in its unphosphorylated state, it exhibits activity in selectively targeting mRNA for quick degradation. Additionally, unphosphorylated TTP demonstrates a higher affinity for ARE-RNA sequences compared to phosphorylated TTP.[26] TTP is up-regulated during inflammatory responses to decrease pro-inflammatory gene expression.[30] An example of TTP's function is its ability to inhibit the synthesis of TNF-α by destabilizing TNF-α mRNA and promotes the degradation of mRNAs encoding multiple inflammatory cytokines.[31] Much less is known about the contribution of RNA-protein interactions to human disease. The fact that various RBPs are involved in glomerular and tubular kidney pathologies is unsurprising. Indeed, it seems likely that RBPs have roles in all kidney diseases. RBPs have been shown to have roles in tubular and glomerular kidney diseases, including AKI, CKD, DKD, kidney fibrosis, polycystic kidney disease (PKD), and glomerulonephritis (GN). RBPs can have both protective and pathogenic roles in kidney diseases; for example, two of the best studied RBPs HuR and YBX1 ameliorate damage in AKI but promote kidney fibrosis.[32]

2. METHODOLOGY

A total of 150 subjects were included in this study, divided into five groups with 30 subjects in each. Four groups consisted of 30 patients of CKD, AKI, DN and nephrotic syndrome (NS). Additionally, 30 healthy individuals without any history of cardiovascular disease (CVD), diabetes mellitus (DM), hypertension, renal diseases, endocrine problems, metabolic disorders, infections, or acute or chronic illnesses were included as the control group. These healthy subjects visited the hospital for routine check-ups. These subjects were enrolled in the study between October 2022 and April 2023 at Imam Al-Sadiq Hospital and Marjan Medical City in Babil province, specifically in Al-Hillah city, Iraq.

The laboratory test analyses were performed. Body mass index (BMI) was calculated. General data, such as age, gender, and medical history, were recorded for each participant. Written informed consent was obtained, and the study methods were approved by both the Ethical Committee of the College of Medicine, University of Al-Qadisiyah, and the Ministry of Health.

A 5 mL blood sample was collected from all the study groups. From this sample, 0.5 ml of blood was promptly transferred into dipotassium-EDTA Vacutainer® tubes for complete blood count (CBC) analysis. The WBC, PCV, neutrophil and lymphocyte counts and neutrophils/lymphocytes ratio (NLR) analysis were performed directly using the Haematology Analyser CBC (Sysmex, Japan), while 0.5 ml of the blood was put in EDTA tubes and 0.5 ml of TRIzol® added. The tubes were then kept at -80 °C for measuring the expression of anti-inflammatory RNA binding protein, Tristetraprolin (TTP) by Quantitative Real Time-Polymerase Chain

Table 1: Demographic characteristics of patients with kidney diseases and control subjects

Variables	CKD (n = 30)	AKI (n = 30)	DN (n = 30)	NS (n = 30)	Control (n = 30)	P-value
Age (y)						
Mean ± SD	58.33 ± 18.33	54.48 ±18.10	60.84 ± 12.38	52.54 ± 14.02	55.06 ± 8.03	0.451 NS
Range	32-73	34-75	28-73	24-76	25-58	
BMI (kg/m^2)						
Mean ± SD	24.5 ± 3.8	25.3 ± 2.6	26.78 ± 3.2	25.79 ± 4.05	24.79 ± 3.34	0.321 NS
Range	14.24-30.21	12.34-31.7	14.14-32.1	31.74 ± 17.95	17.95-28.74	
Gender						
Male	14 (46.6)	15 (50)	12 (40)	17 (56.67)	(11 (36.67)	0.23 NS
Female	16 (53.3)	15 (50)	(18 (60)	13 (20.43)	19 (63.33)	
Family history with kidney diseases						
Yes	5 (16.67)**	7 (3.34)**	11(36.67)**	2 (6.67)**	0	P < 0.01
No	25 (83.34)	23 (76.67)	19 (63.33)	28 (93.33)	30 (100)	0.41 NS

N: numeral of subjects; NS: not significant; Data presented as mean ± SD, Range or n (%)

Reaction (RT-qPCR) by using GoTaq®1-Step RT-qPCR (Promega, USA). After clotting for 30 min, the remaining 4 ml of blood was subjected to centrifugation at 4000 rpm for 15-20 min at room temperature separating the serum. The separated serum was carefully preserved in eppendorf tubes and stored at -20°C for subsequent biochemical analysis. The quantification of S100 A8/A9 was performed using a ELISA method following the instructions provided by the manufacturer (Bioassay BT, China).

Statistical analysis

The data are reported as means ± standard deviation (SD). Statistical analysis was performed using SPSS version 23. Qualitative variables were expressed as numbers and percentages, and the difference between groups was assessed using the Chi-square test. The normality of the data was evaluated using the Andersen-Darling test. To identify significant differences between control and experimental subjects, the Student's t-test was utilized. Additionally, for determining significant differences between multiple groups, a one-way analysis of variance (ANOVA) was performed, followed by post hoc analysis using Tukey's test. A level of $P \leq 0.05$ was considered statistically significant throughout the analysis.

3. RESULTS

We enrolled 150 subjects with 30 patients each; 4 groups consisted of renal disease patients and a healthy control group consisting of 30 subjects. The average age of the control group was (55.06 ± 8.03 y). All clinical and hemodynamic variables are summarized in Table 1.

No significant differences were observed in man age of all patient groups compared to control ($P = 0.451$). Also non-significant changes in BMI were found between patient groups and the control group ($P = 0.321$) (Table 1).

3.1. WBC, neutrophil counts, lymphocytes counts and NLR

The hematological characteristics between individuals with renal disease and the healthy group are summarized in Table 2. NLR was calculated as a marker of inflammatory responses in patients with kidney diseases. A significant increase was indicated in WBC counts in AKI and DN patient groups compared to control group ($P \leq 0.05$). PCV values were decreased significantly in CKD and DN groups in comparison with control ($P \leq 0.05$, Table 2). Significant increase was observed in neutrophil counts in patients with AKI and DN compared to control ($P \leq 0.05$, Table 2). The results show a significant reduction in lymphocyte counts ($P \leq 0.05$) in CKD, DN, and NS ($P \leq 0.05$). Also, the results indicate that NLR is significantly high in all patient groups compared to the control group ($P < 0.05$).

3.2. Serum protein S100A8/A9 levels

The activation of calcium binding protein S100A8/A9 is involved in renal damage. The findings of this study demonstrated a significant increase in the levels of S100A8 in patients with CKD, AKI, NS, and DN compared to the healthy control group ($P \leq 0.05$, Figure 1); higher levels were observed in CKD and AKI.

Table 2: WBC, PCV, neutrophil counts, lymphocyte counts and NLR in kidney diseases and control groups

Variables	CKD (n = 30)	AKI (n = 30)	DN (n = 30)	NS (n = 30)	Control (n = 30)	P-value
WBCs X109/L						
Mean ± SD	9.72 ± 4.4	10.96 ± 3.94*	11.39 ± 4.9*	8.66 ± 2.44	6.7 ± 1.57	< 0.05
Range	4.1-16	-6.5-19	7-15.2	5-15	4.7-8.6	
PCV						
Mean ± SD	29.77 ± 5.24*	32.16 ± 4.38	28.59 ± 5. 41*	37.01 ± 5.9	42.9 ± 5.1	< 0.05
Range	18-38	24-40	18-37	28-47	35-52	
Neutrophils X109/L						
Mean ± SD	6.52 ± 1.93	14.74 ± 7.74*	11.67 ± 4.25*	7.32 ± 2.179	5.86 ± 0.94	< 0.05
Range	4.4-10.4	6-41.6	16.6-6.3	4.5-16.5	4.1-6.9	
Lymphocyte X109/L						
Mean ± SD	1.22 ± 0.4*	1.97 ± 0.77	1.31 ± 0.47*	1.70 ± 0.52*	2.7 ± 0.51	< 0.05
Range	0.6-1.8	0.6-3.16	2.66±0.58	0.9-2.7	2.2-4.3	
NLR						
Mean ± SD	5.54 ± 1.43*	7.95 ± 1.91*	9.64 ± 2.1**	4.6 ± 1.4*	2.26 ± 0.66	< 0.05
Range	3.3-8.2	3.2-9.4	5-19.9	2.8 ± 8	1.3-3.2	

**P < 0.05; ** P < 0.01 NS: not significant*

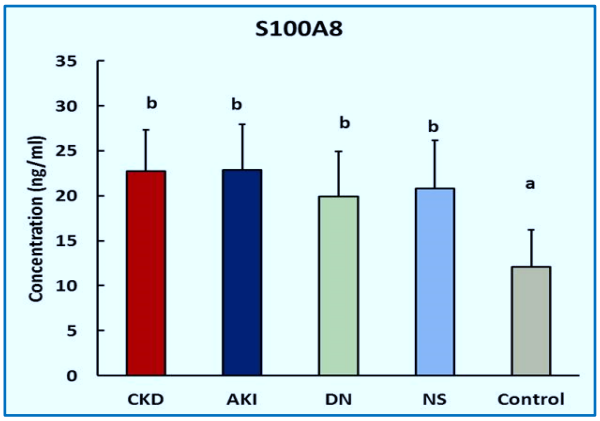

Figure 1: Serum S100A8 levels in patients with CKD, AKI, DN, NS, and control groups. The mean ± SD values are presented in the data. Significant differences between patients and control group were indicated by different letters (P ≤ 0.05)

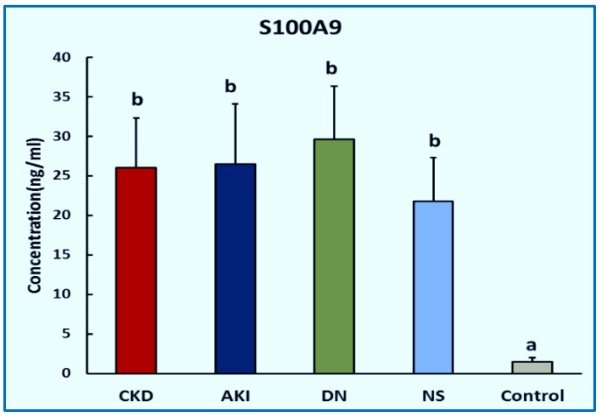

Figure 2: Serum S100A9 levels in patients with CKD, AKI, DN, NS, and control groups (mean ± SD). Significant differences between patients and the control group indicated by different letters (P ≤ 0.05).

In line with S100A8, Figure 2 show that S100A9 protein was increased significantly in all patient groups in comparison to healthy people ($P \leq 0.05$). High level was indicated in CKD, AKI and DN groups in contrast to NS group ($P \leq 0.05$).

3.3. Tristetraprolin (TTP) gene expression in groups

The activity of anti-inflammatory RNA-binding protein, TTP is regulated during the inflammatory response, therefore to indicate whether the TTP is contributed to the regulation of inflammatory events in kidney disease. TTP gene expression was assessed by RT-qPCR. RNA was extracted from blood; The RNA expression in different groups was determined using RT-qPCR, and expression was normalized against endogenous controls. Gene expression is presented as a fold change in expression compared to the control group. The results demonstrated a significant down regulation in the expression of TTP in DN and NS groups compared to the significant increase in TTP expression in comparison to control and other patient groups ($P \leq 0.01$). Non-significant decrease in TTP gene expression was

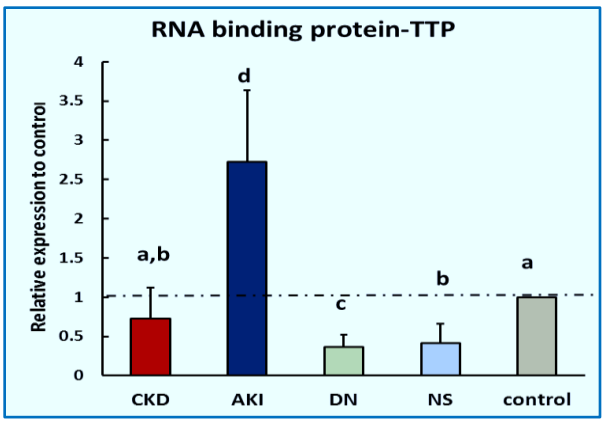

Figure 3: TTP expression by patient groups and control. The data represents means ± SD. Significant differences versus control are denoted by different letters (P ≤ 0.01)

indicated in CKD compared to healthy (P ≥ 0.05). control group (P ≤ 0.01, Figure 3). AKI showed highly significant increase in TTP expression in comparison to control and other patient groups (P ≤ 0.01). Non-significant decrease in TTP gene expression was indicated in CKD compared to healthy group (P ≥ 0.05).

4. DISCUSSION

4.1. Demographic characteristics

All groups aged from 24 to 76 y. There was no statistically significant difference in the mean age and body mass index between groups that conformed to earlier reports about renal disease. Family history was statistically significant in each patient group when compared with the control group (P ≤ 0.05). Family history is a significant risk factor for kidney disease because many kidney diseases have a genetic component. This means that certain genes can be passed down from parents to their children, increasing the likelihood of developing kidney disease such as PKD, IgA nephropathy and Alport syndrome.[33] In these conditions, mutations in specific genes can lead to kidney damage and disease. Also shared environmental and lifestyle factors can contribute to kidney disease like DM, hypertension (risk factors for kidney disease).[34]

4.2. WBCs, PCV, neutrophil, lymphocyte counts and NLR

The study shows significant changes in WBC, PCV, neutrophils / lymphocyte counts and high NLR among patient groups. NLR was once thought to be a marker for endothelial dysfunction and inflammation. It is now thought to be an inflammatory biomarker that reflects both the innate response, which is mediated by neutrophils, and the adaptive response, which is mediated by lymphocytes, and it is stable and less affected by pathological status.[35] The measurement of NLR is both cost-effective and practical for assessing patients with NS, making it a potential alternative to more expensive inflammatory biomarkers.[36] In renal disorders, a high NLR has been associated with the deterioration of renal function in CKD, while an NLR value greater than 1.5 is linked to both early-stage and advanced CKD.[37] NLR is also being recognized as a prognostic marker for the presence and severity of proteinuria in CKD.[38]

AKI involves a complex immune-pathogenesis, which includes the interaction of various factors such as damage-associated molecular patterns (DAMPs), pathogen-associated molecular patterns (PAMPs), ROS, hypoxic-ischemia (HI), the complement system, dendritic cells, neutrophils, lymphocytes, macrophages, and cytokines. As a result, there is a significant increase in WBC count.[39]

Neutrophils, also known as polymorphonuclear cells (PMNs), are crucial innate immune mediators that rapidly respond to pathogens in areas of tissue injury.[40] In AKI, neutrophils enter the kidney and contribute to microvascular obstruction by attaching to the endothelium through adhesion proteins like P-selectin, intracellular adhesion molecule-1 (ICAM-1), and vascular adhesion protein-1 (VAP-1). This attachment leads to the release of cytokines, ROS, and protease enzymes, causing renal damage. Neutrophils have been extensively studied in the context of AKI due to their role as "first responders.[41] Inhibiting VAP-1 or the leukotriene B4-leukotriene B4 receptor axis has shown protective effects against ischemia-reperfusion injury (IRI) and cisplatin-induced AKI, respectively.[42]

In DN, inflammation plays a critical role in the disease's pathogenesis.[43] Various inflammatory chemicals, such as interleukins (ILs) IL-6, IL-18, and TNF-α, have been associated with DN and prolonged inflammation.[35] A sensitive biomarker of inflammation, a total and differential WBC count, is straight forward to perform in the lab.[44] Measuring total and WBC counts serves as a sensitive biomarker for inflammation and can be easily performed in the laboratory. In DN and other disorders, neutrophilia and lymphocytopenia are independent markers, and the majority of WBCs play a significant role in the chronic meta-inflammatory states observed in DN. The rise in neutrophil counts is primarily responsible for the increased WBC count in DN.[45]

4.3. S100 A8 /A9 Ca2+–binding protein levels

When leukocytes invade the kidney, S100A8/A9 proteins mediate renal scarring, and the death of tubular cells. By causing loss of cell junction proteins and stopping cellular growth, study results showed that levels of S100A8 were increased significantly in patients

with CKD, AKI, NS and DN, compared to the control group ($P \leq 0.05$). Significantly high levels of S100A9 were also observed in each patient group compared to healthy subjects ($P \leq 0.05$).

The development of renal fibrosis, a hallmark of CKD, is facilitated by the release of DAMPs following tissue damage and the innate immune receptors implicated in their detection.[46] S100A8/A9 change the shape of the tubular epithelial cells (TECs) in a bad way and may be the cause of any permanent tubular damage that happens later. When S100A8 and S100 A9 proteins are paired with a profibrotic trigger like TGF-1β, such alterations in tubular morphology eventually leads to cell death via apoptosis. To stop renal fibrosis and parenchymal damage in CKD patients, a potential therapeutic approach is to target the S100A8 and S100 A9 proteins, TLR-4 and RAGE ligands.[47] These proteins promote tubular apoptosis and renal fibrosis by causing the loss of connections between TECs and stimulating the epithelial-to-mesenchymal transition (EMT) process.[47]

In DN, the levels of S100A8 and S100A9 proteins are significantly increased in TECs of diabetic kidneys. Overexpression of these proteins leads to the development of renal interstitial fibrosis in diabetic mice. In diabetes, elevated levels of S100A8 and A9 proteins in TECs stimulate the TLR4/NF-κβ signalling pathway, enhance the EMT process, and ultimately contribute to the progression of renal interstitial fibrosis. A potential therapeutic approach for treating DN may involve using the tiny molecular inhibitor AB38b to block the unusual expressions of S100A8 and A9.[14]

NS is characterized by increased production of S100A8 and S100A9 proteins in the kidneys' glomeruli. The exact mechanism by which these proteins contribute to NS's pathogenesis is not fully understood. However, it is believed that they may activate and recruit inflammatory cells like neutrophils and macrophages in the kidneys and produce cytokines, such as IL-1β and TNF-α, which further intensify the inflammatory response. Additionally, S100A8 and S100A9 may also play a role in the development of proteinuria by interacting with podocytes, which increases permeability and leakage of protein into the urine.[48]

4.4. TTP gene expression level

TTP regulates the expression of mRNAs containing AU-rich elements in a significant manner. The results demonstrated a significant down regulation in the expression of TTP in CKD, DN and NS groups compared to the control ($P \leq 0.01$). These findings indicate that diabetes with clinical proteinuria is associated with a lowered serum amount of TTP and elevated levels of IL-6 and IL-18. TTP expression reduction may precede IL-6 and IL-18 elevation and may provide an earlier predictor for glomerular impairment than IL-6 and IL-18.[30] TTP is an ARE-binding protein with cell type-specific expression characteristics that regulates diverse target mRNAs to exert distinct functions. TTP attaches to the AREs of multiple cytokine-encoding mRNAs and promotes their degradation. We previously demonstrated that serum levels of TTP are lower in patients with diabetes and clinical proteinuria compared to healthy individuals, suggesting that TTP may negatively regulate the progression of DKD.[31,49]

The levels of STAT6 mRNA expression were found to be significantly increased and positively correlated with an early unfavorable progression of NS. Approximately one-third of patients with minimal change disease (MCD) exhibit STAT6 activation upon exposure to interleukin-13 (IL-13). The IL-13/STAT6 signaling pathway appears to play a crucial role in NS by promoting STAT6 phosphorylation and its translocation into the nucleus, thereby exacerbating kidney damage. In vitro experiments involving the overexpression of tristetraprolin (TTP) resulted in the inhibition of IL-13/STAT6 signaling. This suggests that TTP may down-regulate the IL-13 signaling system, providing a protective effect against kidney damage.[50]

In AKI, there is a rapid and robust inflammatory response that contributes to the pathogenesis of the disease. This study has shown a highly significant increase in TTP gene expression in the kidneys compared to control and other patient groups ($P \leq 0.01$). The up-regulation of TTP in AKI is thought to be an adaptive response to limit the excessive inflammatory response that occurs in the disease. Cytokines, such as TNF- α, IL-1 beta, and IL-6, are known to be involved in the pathogenesis of AKI. These cytokines can activate signaling pathways that lead to the expression of pro-inflammatory genes and the secretion of additional cytokines and chemokines, which can further exacerbate the injury.[50]

5. CONCLUSION

The current study results indicate that neutrophil-to-lymphocyte ratio in blood is a promising inflammatory sign indicating the severity of renal diseases. Neutrophil-to-lymphocyte ratio is considered a less expensive and effective measure of inflammation. The current results showed that levels of S100A8/A9 were up-regulated in patients with renal diseases compared to the control group. Interestingly, a significant increase was found in in anti-TTP mRNA expression with down regulation in renal diseases, which contributed to compensatory effects as pro-inflammatory cytokine production and lowered TTP concentrations leading to persistent inflammation.

6. Data availability

The numerical data generated during this research is available with the authors.

7. Conflict of interest

The study utilized the hospital resources only, and no external or industry funding was involved.

8. Authors' contribution

All authors took equal part in the conduct of the study, literature search, and preparation of the manuscript. All authors have read the final manuscript and endorse it.

9. REFERENCES

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