ONLINE FIRST

ORIGINAL ARTICLE

Evaluating the levels of Receptors for Advanced Glycation End Products in Focal Segmental G*lomerulosclerosis*

Habib-E-Ajmi[1], Zulfiqar Ali[2]*, Tahir Maqbool[1], Amtul Huda[3], Nida[4], Faiza Habib[5]

[1]*Centre of Research in Molecular Medicine, Institute of Molecular Biology and Biotechnology, The University of Lahore, Lahore, Pakistan*
[2]*Department of Pathology, Rashid Latif Khan University Medical College, Lahore, Pakistan*
[3]*Department of Physiology, Abbottabad International Medical and Dental College, Abbottabad, Pakistan*
[4]*Department of Physiology, Avicenna Medical College, Lahore, Pakistan*
[5]*Department of Physiology, CMH Lahore Medical College & Institute of Dentistry, Lahore, Pakistan*
Correspondence: *dr.zulfiqarali53@gmail.com*
doi: 10.22442/jlumhs.2026.01393

ABSTRACT

OBJECTIVE: Serum levels of EN-RAGE, sRAGE, the ratio of EN-RAGE/sRAGE, and serum GSH in subjects with FSGS are comparable to age- and gender matched controls and may be implicated in RAGE-mediated inflammation and oxidative stress in FSGS.

METHODOLOGY: A case–control study was conducted on 40 focal segmental glomerulosclerosis patients and 40 age- and gender-matched healthy controls after approval from the Institutional Review Board (Ref-IMBB/BBBC/24/1400-A). Serum GSH, EN-RAGE, and sRAGE were quantified by ELISA. We also assessed demographic parameters, including age, gender, height, BMI, and gender orientation.

RESULTS: Patients with focal segmental glomerulosclerosis showed significantly higher EN-RAGE and EN-RAGE/sRAGE ratio compared to controls ($p \leq 0.05$). GSH levels and demographic variables did not differ significantly between groups.

CONCLUSION: Elevated EN-RAGE/sRAGE and reduced antioxidant markers suggest that RAGE pathway activation and oxidative stress contribute to focal segmental glomerulosclerosis pathogenesis. Targeting these mechanisms may provide novel therapeutic opportunities.

KEYWORDS: Focal segmental glomerulosclerosis, kidney disease, sRAGE, EN-RAGE.

INTRODUCTION

In the human body, the kidney is an organ responsible for excreting metabolic waste products and maintaining water balance. The kidneys are two organs in the shape of beans that are a fundamental component of the human body's balancing mechanism. They are situated in the retroperitoneal area, just underneath the ribs. They are involved in a variety of processes, including bloodstream filtration, metabolic regulation, and maintenance of water balance[1]. The kidneys' diverse morphology and functioning are vital to the human organism's general well-being and health. Inflammation and immunity are increasingly linked to receptors for advanced glycation end products (RAGE) and its soluble forms[2]. The hydrophobic receptor RAGE binds a wide range of ligands, including compounds derived from infections and injured cells in mammals, adhesion proteins, and advanced glycation end products, generating intracellular messages that activate pro-inflammatory pathways. The receptor (RAGE) remains constitutively expressed at elevated levels in the epidermis or airways of healthy humans. Still, it is increased in multiple types of cells throughout the body in cardiometabolic and inflammatory illnesses[2]. The glomeruli, the kidney's filtering components, become inflamed when a person has glomerulonephritis. Autoimmune conditions, illnesses, or certain drugs may trigger it. Urine that contains blood or protein, edema (swelling), and elevated blood pressure are potential warning signs[3]. According to Wang Y 2024[4], an afferent arteriole permits blood to enter the glomerulus, whereas the efferent arteriole permits plasma to leave.

Notably, sRAGE levels have recently been linked to insulin resistance and other elements of the MetS, such as proinflammatory states, high blood pressure, and atherogenic dyslipidemia. The extracellularly discovered receptor for advanced glycation end products binding protein (EN-RAGE) is a proinflammatory protein that binds to calcium and is mostly released by lymphocytes, macrophages, and activated granulocytes. One form of DAMP that is produced extracellularly is EN-RAGE. It binds to RAGE, activates intracellular lymphocyte signaling cascades, and causes the production of proinflammatory cytokines, thereby inducing a prolonged inflammatory-immune response. Numerous inflammatory-immune diseases have been linked to elevated serum EN-RAGE levels. The urinary tract and kidneys are extremely susceptible to oxidative stress damage due to their elevated energy expenditure, exposure to contaminants, and the production of toxic metabolic byproducts. Reduced Glutathione concentrations in focal segmental glomerulosclerosis could make it more difficult for the renal system to combat oxidative stress-related damage, potentially worsening the condition and contributing to dysfunction[5].

Variations in Glutathione concentrations among kidney failure patients could serve as indicators of disease progression. Several investigations on patients suffering from renal failure have identified lower levels of glutathione, especially while experiencing illness symptoms or instances associated with elevated stress from oxidative damage. Approaches that increase GSH levels or enhance its antioxidant ability are currently being researched as possible treatments for focal segmental glomerulosclerosis. These include dietary changes, multivitamin supplements, endogenous anti-inflammatory delivery, and glutathione synthesis. To determine the safety and efficacy of these prescription drugs in healthcare settings, more research is needed. Focal segmental glomerulosclerosis is closely associated with oxidative stress and inflammatory processes that contribute to progressive renal damage. The EN-RAGE/sRAGE system plays a key role in mediating these pathological events through the RAGE signaling pathway[6]. However, clinical evidence on circulating levels of EN-RAGE and sRAGE and their relationship with antioxidant status in focal segmental glomerulosclerosis patients remains limited. Evaluating

these serum biomarkers may provide insight into disease mechanisms and support their potential use in monitoring disease progression and therapeutic response.

Focal segmental glomerulosclerosis (FSGS) is a progressive glomerular disease that may involve inflammatory pathways and oxidative stress. The role of the EN-RAGE/sRAGE axis and antioxidant protection in FSGS is poorly understood. The assessment of these biomarkers can help to gain insight into the mechanisms leading to disease progression and their possible clinical relevance.

METHODOLOGY

The study was conducted at the University of Lahore, Lahore, Pakistan, after approval from the Institutional Research Board, IMBB, University of Lahore. Each participant was screened at Lahore's most renowned Govt. Jinnah Hospital. The study included 80 volunteers, and informed consent was obtained before the study began. Inclusion criteria: Adults aged 30–50 years, renal biopsy-confirmed focal segmental glomerulosclerosis, newly diagnosed or clinically confirmed focal segmental glomerulosclerosis patients, patients who provided written informed consent. Exclusion criteria: Diabetes mellitus, Chronic liver disease, Malignancy, Autoimmune diseases, Acute infections, Individuals with other renal diseases. These subjects were divided into two distinct categories: 40 individuals diagnosed with focal segmental glomerulosclerosis for evaluation, while the remaining 40 were healthy.

To choose the research participants, an efficient, convenient non-probability sampling method was used. Ages ranged from 30 to 50 years. The research excluded individuals with diagnosed conditions or those taking medications that might interfere with the results. The control group received no medication. Urine dipsticks were used for analysis. An automated Sysmex XP-2100 haematology analyzer was used to perform a complete blood count on the selected patients. The human-accessible diagnostic ELISA kit (Zokeo) was used to quantify the levels of sRAGE and EN-RAGE. Briefly, serum samples and standards were added to antibody-coated microplates, incubated, washed, and treated with enzyme-conjugated detection antibodies. Following substrate addition and reaction termination, absorbance was measured at 450 nm using a microplate reader, and the concentrations of GSH, sRAGE, and EN-RAGE were calculated from the respective standard curves.

Statistical Analysis: Data were analyzed using GraphPad Prism version 8. We assessed the normality of continuous variables using the Shapiro–Wilk test. We compared FSGS patients and healthy controls using the unpaired Student's t-test for normally distributed variables. Results are expressed as mean ± SD. A p-value <0.05 was considered statistically significant.

RESULTS

Demographic and Biochemical Profile

This study investigated clinical and laboratory findings between healthy controls and the focal segmental glomerulosclerosis group, revealing clear biochemical distinctions. The study particularly examined two markers, soluble receptor for advanced glycation end products (sRAGE) and extracellular nuclear receptor for advanced glycation end products (EN-RAGE), to evaluate their potential as indicators of disease status. The total number of individuals recruited into the study was 80: 40 patients and 40 healthy controls. All the participants in the current study were matched for age, sex, and body mass Index. **Table I** summarizes the demographic profile of the patients and controls.

Table I: Comparison of biochemical and hematological parameters between the control group (n = 40) and the focal segmental glomerulosclerosis group (n = 40).

Variable	Control (n=40)	Focal segmental glomerulosclerosis (n=40)
Urea	35.00±0.00	47.20±8.044
Creatinine	1.200±0.00	3.160±0.4159
Hemoglobin	13.00±0.00	11.17±1.786
MCHC (%3)	35.00±0.00	31.00±1.000
RBCs (x106 /uL)	6.000±0.00	4.180±0.9985
WBCs (x103 /uL)	11.00±0.00	8.460±2.294
MCH	32.00±0.00	24.80±3.114
HCT (%)	52.00±0.00	33.20±5.891
Neutrophils (%)	75.00±0.00	67.80±9.550
Lymphocytes (%)	45.00±0.00	27.00±6.245
Monocytes (%)	10.00±0.00	7.000±1.581
Eosinophil (%)	6.000±0.00	2.400±0.5477
MCV	96.00±0.00	78.80±12.34
Platelets	450.0±0.00	254.2±33.48

All values are expressed as mean ± standard deviation to illustrate the magnitude and direction of differences between the two cohorts.

Receptors for Advanced Glycation End Products (RAGE) in Focal Segmental Glomerulosclerosis

In the comparative analysis of receptor for advanced glycation end products (RAGE) isoforms between focal segmental glomerulosclerosis subjects and healthy matched controls, we observed significant elevations in both soluble RAGE (sRAGE) and nuclear RAGE (EN-RAGE) levels among patients. Focal segmental glomerulosclerosis subjects exhibited markedly higher sRAGE concentrations, aligning with the pathological involvement of advanced glycation end products (AGEs) and their receptors in chronic kidney disease. The elevated sRAGE may reflect an upregulated systemic response to oxidative stress and inflammation, acting as a decoy receptor to sequester circulating AGEs and thereby limit their interaction with membrane-bound RAGE, ultimately reducing pro-inflammatory signaling. In parallel, EN-RAGE levels were also significantly increased in focal segmental glomerulosclerosis patients compared to healthy

controls. This upregulation of EN-RAGE, which plays a role in intracellular signaling pathways that mediate fibrosis and inflammation, further indicates active disease processes within the kidney tissue. The lower levels of both sRAGE and EN-RAGE in healthy controls suggest a stable biochemical and cellular environment devoid of such pathological stimuli. Collectively, these findings emphasize the distinct biochemical profile in focal segmental glomerulosclerosis and support the potential of both sRAGE and EN-RAGE as valuable biomarkers for disease activity, severity, and therapeutic monitoring in chronic kidney disease. As shown in Table 2 and **Figure 1**. In addition, we evaluated serum antioxidant status by measuring GSH levels. Patients with focal segmental glomerulosclerosis exhibited significantly lower GSH concentrations than healthy controls, indicating impaired antioxidant defense and increased oxidative stress associated with disease progression (**Table II**).

Table II: Comparison of serum GSH, sRAGE, and EN-RAGE concentrations between healthy controls and patients with focal segmental glomerulosclerosis

		Control	Patients
1	GSH	2.15±0.21	1.0±0.02
2	s Rage	2.774±0.5343	8.266±0.7676
3	EN-RAGE	3.052±1.586	9.912±0.6742

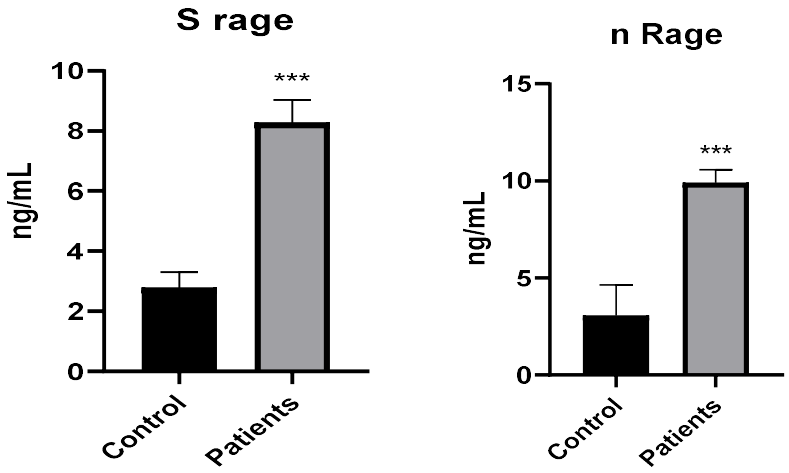

Figure 1: Graphical Presentation of sRAGE and EN-RAGE among the study group, showing a significant difference between the control and diseased groups. Data of the experimental group are expressed as mean ± SD, where $P \leq 0.05$.

DISCUSSION

The present study demonstrates that patients with focal segmental glomerulosclerosis exhibit significantly increased circulating levels of sRAGE and EN-RAGE compared with healthy controls, alongside diminished antioxidant defenses (GSH). These findings provide new insights into the role of RAGE-mediated signaling and oxidative imbalance in the pathogenesis of chronic liver disease. RAGE acts as a pattern recognition receptor that has been linked to an assortment of fibrous and persistent inflammatory disorders, which was the primary objective of the research conducted[7].

Previous studies have reported similar patterns of RAGE system activation in chronic kidney disease and diabetic nephropathy, where EN-RAGE overexpression promotes cytokine production, macrophage recruitment, and extracellular matrix accumulation. The increased sRAGE concentration in this study might represent a compensatory response aimed at neutralizing circulating ligands and minimizing RAGE-mediated inflammation[8]. However, the predominance of EN-RAGE over sRAGE indicates that this compensatory mechanism is insufficient to counteract ongoing injury. Such a pattern has also been described in other fibrotic diseases, suggesting a shared inflammatory pathway across organ systems[9].

The reduction of antioxidant markers further supports the role of oxidative stress in focal segmental glomerulosclerosis. Depletion of GSH reflects excessive production of reactive oxygen species, which perpetuate lipid peroxidation, protein oxidation, and DNA damage. Reactive oxygen species can also upregulate RAGE expression, thereby creating a self-sustaining cycle of oxidative stress and inflammation. This bidirectional interaction between RAGE signaling and oxidative imbalance may underlie progressive glomerular scarring and functional deterioration in nephrotic patients[10].

Clinically, these observations suggest that circulating EN-RAGE, sRAGE, and antioxidant markers could serve as potential non-invasive indicators of disease activity. Measurement of these biomarkers may help identify patients at risk of rapid progression and guide therapeutic decision-making[11]. Moreover, pharmacological modulation of RAGE signaling or antioxidant supplementation could represent novel strategies to halt or slow renal fibrosis[12]. The promising outcomes of RAGE antagonists in experimental models of diabetic nephropathy further support this therapeutic potential[13].

The present study, however, has certain limitations. The sample size was modest, and the cross-sectional design restricts causal interpretation. Only serum biomarkers were analyzed[14], and tissue-level expression or gene regulation of RAGE was not evaluated. In addition, the study did not assess correlations with clinical parameters such as glomerular filtration rate or proteinuria. Future studies with larger cohorts, longitudinal follow-up, and combined molecular approaches are warranted to validate these findings and clarify the temporal relationship between RAGE activation, oxidative stress, and renal injury[14,15].

The current study provides evidence that activation of the EN-RAGE/sRAGE axis and suppression of antioxidant defense mechanisms contribute to the pathophysiology of focal segmental glomerulosclerosis. The interplay between oxidative stress and RAGE signaling appears central to disease progression[16]. These findings highlight potential diagnostic and therapeutic implications and encourage further investigation into RAGE-targeted and antioxidant-based interventions to preserve renal structure and function. The present findings indicate that circulating sRAGE and EN-RAGE may serve as promising biomarkers for monitoring disease activity in focal segmental glomerulosclerosis[17]. Future multicenter studies with larger patient populations and longitudinal follow-up are needed to determine their

prognostic value and relationship with renal function decline. Furthermore, mechanistic studies investigating RAGE-mediated signaling pathways may facilitate the development of targeted therapeutic strategies aimed at reducing inflammation and oxidative stress in focal segmental glomerulosclerosis[18]. Collectively, this study expands current knowledge regarding the involvement of the RAGE axis in focal segmental glomerulosclerosis and supports further investigation of these biomarkers in clinical practice.

CONCLUSION

Serum levels of sRAGE and EN-RAGE were significantly elevated in focal segmental glomerulosclerosis patients, while antioxidant markers (GSH) were reduced. This indicates activation of RAGE-mediated inflammatory pathways and impaired antioxidant defense, suggesting their potential as biomarkers for focal segmental glomerulosclerosis.

Ethical Permission: University of Lahore, Pakistan ERC approval letter No. IMBB/BBBC/24/1400-A.
Conflict of interest: There is no conflict of interest between the authors.
Financial Disclosure / Grant Approval: No funding agency was involved in this research.
Data Sharing Statement: The corresponding author can provide the data proving the findings of this study on request. Privacy or ethical restrictions prevented us from sharing the data publicly.

AUTHOR CONTRIBUTION

Ajmi HE: substantially contributed to the conception and design of the study, data collection, experimental analysis, interpretation of results, and manuscript drafting.
Ali Z: substantially contributed to the conception and design of the study, data collection, experimental analysis, interpretation of results, and manuscript drafting.
Maqbool T: substantially contributed to the conception and design of the study, data collection, experimental analysis, interpretation of results, and manuscript drafting.
Huda A: assisted in data interpretation, literature review, and critical revision of the manuscript for intellectual content.
Nida: assisted in data interpretation, literature review, and critical revision of the manuscript for intellectual content.
Habib F: assisted in data interpretation, literature review, and critical revision of the manuscript for intellectual content.
All authors reviewed and approved the final version of the manuscript.

REFERENCES

1. Miguel V, Shaw IW, Kramann R. Metabolism at the crossroads of inflammation and fibrosis in chronic kidney disease. Nat Rev Nephrol. 2025; 21(1): 39-56.
2. Gasparotto J, Somensi N, Girardi CS, Bittencourt RR, de Oliveira LM, Hoefel LP et al. Is it all the RAGE? Defining the role of the receptor for advanced glycation end products in Parkinson's disease. J Neurochem. 2024; 168(8): 1608-24.
3. Ahmed K, Dubey MK, Dubey S, Pandey DK. Chronic kidney disease: causes, treatment, management, and future scope. In: Computational Intelligence for Genomics Data. Academic Press; 2025. p. 99-111.
4. Wang Y, Fang Y, Vrablik M. Homeostasis model assessment for insulin resistance mediates the positive association of triglycerides with diabetes. Diagnostics. 2024; 14(7): 733.
5. Dragoș D, Enache II, Manea MM. Oxidative stress and nutritional antioxidants in renal diseases: a narrative review. Antioxidants. 2025; 14(7): 757.
6. Kanikowska D, Kanikowska A, Strojny Z, Kawka E, Zawada A, Rutkowski R, Korybalska K. Assessment of EN-RAGE, sRAGE, and its isoforms: cRAGE, esRAGE in obese patients treated by moderate caloric restriction combined with physical activity conducted in hospital condition. Cytokine. 2024; 180: 156665.
7. Zoccali C, Mallamaci F, Lightstone L, Jha V, Pollock C, Tuttle K et al. A new era in the science and care of kidney diseases. Nat Rev Nephrol. 2024; 20(7): 460-72.
8. Dalal D, Singh L, Singh A. Calycosin and kidney health: a molecular perspective on its protective mechanisms. Pharmacol Rep. 2025; 1-12.
9. Liu H, Xiang X, Shi C, Guo J, Ran T, Lin J et al. Oxidative stress and inflammation in renal fibrosis: Novel molecular mechanisms and therapeutic targets. Chem Biol Interact. 2025 Nov 1; 421: 111784. doi: 10.1016/j.cbi.2025.111784. Epub 2025 Oct 17.
10. Zhou M, Zhang Y, Shi L, Li L, Zhang D, Gong Z et al. Activation and modulation of the AGEs-RAGE axis: implications for inflammatory pathologies and therapeutic interventions–a review. Pharmacol Res. 2024; 206: 107282.
11. Kim G, Yoo HJ, Yoo MK, Choi JH, Lee KW. Methylglyoxal-derived hydroimidazolone-1/RAGE axis induces renal oxidative stress and renal fibrosis in vitro and in vivo. Toxicology. 2024; 507: 153887.
12. Zhu D, Ni Y, Chen C, Dong Z, Wang L, Zhang W. Geniposide ameliorates diabetic nephropathy in type 2 diabetic mice by targeting AGEs-RAGE-dependent inflammatory pathway. Phytomedicine. 2024; 135: 156046.
13. Taguchi K, Fukami K. RAGE signaling regulates the progression of diabetic complications. Front Pharmacol. 2023; 14: 1128872.
14. Hou S, Yang B, Chen Q, Xu Y, Li H. Potential biomarkers of recurrent FSGS: A review. BMC Nephrology. 2024; 25(1): 258.
15. Altintas MM, Agarwal S, Sudhini Y, Zhu K, Wei C, Reiser J. Pathogenesis of focal segmental glomerulosclerosis and related disorders. Ann Rev Pathology: Mechanisms of Disease. 2025; 20(1): 329-353.
16. Liu J, Jin Z, Wang X, Jakoš T, Zhu J, Yuan Y. RAGE pathways play an important role in regulation of organ fibrosis. Life Sci. 2023; 323: 121713.
17. Sun Y, Liu S, Ding W, Zhu C, Jiang G, Li H. Recent advances in miRNA biomarkers for diagnosis and prognosis of focal segmental glomerulosclerosis. Kidney Diseases. 2025; 11(1): 283-291.
18. Patera F, Gatticchi L, Cellini B, Chiasserini D, Reboldi G. Kidney fibrosis and oxidative stress: from molecular pathways to new pharmacological opportunities. Biomolecules. 2024; 14(1): 137.