Oxidative Stress and Renal Function Decline in Type 2 Diabetes Mellitus: A Cross-Sectional Study from Samarra General Hospital

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ABSTRACT

Background: Stroke remains a leading cause of death and long-term disability globally, with traditional rehabilitation approaches primarily focusing on compensatory strategies rather than neural repair mechanisms.

Objective: This narrative review synthesizes current evidence on neuroplasticity mechanisms underlying stroke recovery and evaluates therapeutic interventions that harness the brain's reorganization capacity to improve functional outcomes.

Methods: A comprehensive literature search was conducted using PubMed, Embase, and Cochrane Library databases for English-language studies published between 2000 and April 2023. Search terms included "stroke rehabilitation," "neuroplasticity," "motor recovery," and specific interventions.

Results: Key neuroplastic mechanisms—including synaptic plasticity, dendritic remodeling, cortical reorganization, neurogenesis, and axonal sprouting—underlie both spontaneous and therapy-induced recovery. Evidence-based interventions leveraging these mechanisms include constraint-induced movement therapy (CIMT), physical exercise, non-invasive brain stimulation, virtual reality training, brain-computer interfaces, and emerging cell-based therapies. Recovery outcomes are significantly influenced by individual factors (age, genetics, stroke characteristics) and contextual factors (rehabilitation timing, intensity, resource availability).

Conclusions: Neuroplasticity-based rehabilitation represents a paradigm shift from compensatory to restorative approaches. Future directions emphasize early, intensive, personalized interventions combining behavioral, neuromodulatory, and pharmacological strategies to optimize functional recovery and quality of life for stroke survivors.

KEYWORDS : Modulator, IL_33, ELISA .

INTRODUCTION

*T*ype 2 diabetes mellitus (T2DM) is a highly prevalent metabolic disorder characterized by chronic hyperglycemia and multisystem complications. In 2021, an estimated 537 million adults aged 20–79 years—10.5% of this age group—were living with diabetes worldwide, a figure projected to rise to 783 million by 2045 [1]. The expanding diabetes burden is mirrored by escalating macrovascular (coronary artery disease, stroke, peripheral arterial disease) and microvascular complications (retinopathy, neuropathy, nephropathy), which together impose substantial clinical, social, and economic costs [1,2]. Among microvascular sequelae, diabetic nephropathy (DN)—or diabetic kidney disease (DKD)—is particularly consequential: it develops in roughly 30–40% of people with diabetes and remains a leading cause of chronic kidney disease (CKD) and end-stage kidney disease (ESKD) globally [2,3]. Clinically, DN typically progresses from microalbuminuria to overt proteinuria, accompanied by a declining estimated glomerular filtration rate (eGFR), and may culminate in kidney failure if not optimally controlled [2].

A converging body of evidence implicates oxidative stress (OS) as a unifying mechanism in the pathogenesis and progression of diabetic complications, including DN [4–6]. OS reflects an imbalance favoring reactive oxygen species (ROS) over antioxidant defenses, driven in diabetes by glucose autoxidation, activation of the polyol pathway, protein kinase C (PKC) signaling, and advanced glycation end-product (AGE) formation, alongside depletion or inhibition of endogenous antioxidant systems (e.g., reduced activities of SOD, CAT, GPx, and diminished glutathione) [4–6]. In the kidney, ROS-mediated damage targets glomerular endothelial cells, mesangial cells, and podocytes—disrupting the integrity of the filtration barrier and promoting albuminuria—while pro-inflammatory and pro-fibrotic cascades (notably NF-κB and TGF-β signaling) amplify tissue injury and scarring [4–6].

At the biomarker level, patients with T2DM often exhibit increased lipid peroxidation—frequently indexed by elevated malondialdehyde (MDA)—together with lower activities of key antioxidant enzymes; however, findings are not entirely uniform across studies, reflecting heterogeneity in populations, disease duration, comorbidities, and glycemic control [7–9]. Importantly, data from incipient nephropathy stages and from Middle Eastern cohorts (including Iraq) remain comparatively limited, underscoring the need for context-specific studies that relate oxidative indices to early renal impairment [2,7–9].

Rationale and Objective. Building on this pathophysiologic framework, the present cross-sectional study evaluates oxidative stress markers (e.g., MDA, antioxidant enzymes, total antioxidant capacity) in T2DM patients from Samarra, and examines their associations with renal indices (e.g., eGFR, albuminuria). We hypothesize that patients with T2DM will demonstrate higher oxidative burden and reduced antioxidant defenses relative to healthy controls, and that these abnormalities will correlate with early markers of renal dysfunction, thereby informing targeted antioxidant and reno-protective strategies.

Materials and methods

Apparatus

Study design and setting: This is an original, observational study with a cross-sectional, case-control design. The research was carried out at Samarra General Hospital in Salah ad-Din Governorate, Iraq. Ethical approval was obtained from the hospital's research ethics committee, and all participants provided informed consent prior to enrollment. The study period spanned from [April] 2024 to [April] 2025, during which patients were recruited from the hospital's outpatient diabetes clinics and relevant inpatient departments.

Participants: Two groups were studied – a T2DM patient group and a non-diabetic control group – each consisting of 20 adult individuals. Inclusion criteria for the diabetic group were: adults (age 35–70 years) with established type 2 diabetes mellitus (diagnosed per American Diabetes Association criteria: FPG ≥126 mg/dL or HbA1c ≥6.5%, etc.), under routine follow-up at the hospital. We specifically included patients showing early evidence of nephropathy (incipient renal function decline), as indicated by persistent microalbuminuria or mildly reduced eGFR, but not yet advanced kidney failure. This was reflected in screening measurements of urine albumin-creatinine ratio (ACR ≥30 mg/g but <300 mg/g, corresponding to microalbuminuria) and eGFR generally above 30 mL/min/1.73 m². Exclusion criteria for patients were: type 1 diabetes, overt nephropathy (macroalbuminuria >300 mg/g or end-stage renal disease requiring dialysis), uncontrolled hypertension (>160/100 mmHg) or other significant causes of kidney injury (e.g. glomerulonephritis, urinary tract infection), active infection or inflammatory disorders, and current use of high-dose antioxidant supplements which could skew oxidative stress measurements. The 20 control subjects were healthy volunteers without diabetes (confirmed by normal fasting glucose and HbA1c <5.7%), without hypertension, and with no history of kidney or cardiovascular disease. Controls were age- and sex-matched as closely as possible to the patient group to ensure comparability. All subjects were non-smokers or light smokers and were instructed to avoid any vigorous exercise or unusual dietary antioxidant intake for at least 24 hours before sampling to minimize acute effects on oxidative stress markers.

Clinical and laboratory data collection: Each participant underwent a clinical evaluation including medical history and physical examination. For diabetic patients, duration of diabetes, medications, and presence of any complications were noted. After an overnight fast of 8–10 hours, venous blood samples were collected in the morning from each subject. A portion of blood was collected in plain tubes for serum, and another portion in EDTA tubes for HbA1c analysis. Additionally, a spot midstream urine sample (early morning where feasible) was obtained for measuring albumin and creatinine.

Oxidative stress markers: We assessed a panel of oxidative stress indices in serum:

- **Malondialdehyde (MDA):** MDA levels were measured as an index of lipid peroxidation. We employed the thiobarbituric acid reactive substances (TBARS) assay, in which MDA reacts with

thiobarbituric acid to form a colored MDA-TBA complex measured spectrophotometrically at 532 nm. Results are expressed in nmol MDA per mL of serum.

- **Superoxide dismutase (SOD):** Serum SOD activity was determined using an enzymatic assay based on the enzyme's ability to inhibit the reduction of a tetrazolium salt (for example, the xanthine oxidase/cytochrome c method or a commercial SOD assay kit). SOD activity is reported in U/mL, where one unit is defined as the amount of enzyme inhibiting the rate of reaction by 50% under assay conditions.
- **Catalase (CAT):** Catalase activity in serum was measured by tracking the decomposition of hydrogen peroxide. In a typical assay, H_2O_2 breakdown is monitored at 240 nm, and catalase activity (U/mL) is calculated from the rate of H_2O_2 consumption.
- **Glutathione peroxidase (GPx):** GPx activity was measured via a coupled enzymatic method (e.g. using glutathione reductase and NADPH). The decrease in NADPH absorbance at 340 nm indicates GPx activity as it reduces organic peroxides in the presence of glutathione. GPx activity is expressed in U/L.
- **Total antioxidant capacity (TAC):** The cumulative antioxidant power of serum was evaluated by the Trolox equivalent antioxidant capacity assay or ferric reducing ability of plasma (FRAP) assay. In the TAC assay, antioxidants in the sample reduce a colorimetric substrate (such as ABTS or Fe^{3+}-TPTZ complex), and the change is compared to a standard (Trolox). TAC results are given in mmol/L Trolox equivalents.

All assays were performed in duplicate for accuracy, and we included appropriate calibration standards and quality controls. The laboratory techniques followed manufacturer instructions for assay kits (where used) and were carried out at the hospital's central laboratory with calibrated equipment.

Renal function tests: Serum creatinine was measured by the kinetic Jaffé method (alkaline picrate) on an automated analyzer. Blood urea nitrogen (BUN) was measured by a urease-oxidase method. Estimated GFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation (appropriate for age, sex, and serum creatinine) to standardize kidney function across individuals; eGFR is expressed in mL/min/1.73 m². Urinary albumin and creatinine concentrations were measured from the spot urine. Albumin was measured with an immunoturbidimetric assay (high-sensitivity for microalbumin detection), and urine creatinine by an enzymatic method. The albumin-to-creatinine ratio (ACR) was then calculated (mg albumin per g creatinine) to estimate the degree of albuminuria. An ACR ≥30 mg/g was considered microalbuminuria (incipient nephropathy) and >300 mg/g as macroalbuminuria. For our patient group, ACR values confirmed microalbuminuria on at least two occasions 3 months apart (as per standard definition of persistent microalbuminuria).

Glycemic and lipid profile measurements: Fasting plasma glucose (FPG) was measured by the glucose oxidase-peroxidase method. HbA1c was analyzed using high-performance liquid chromatography (HPLC) (NGSP-certified method) and is reported as a percentage. The lipid profile was determined enzymatically: total cholesterol and triglycerides were measured by standard colorimetric assays, high-density lipoprotein cholesterol (HDL-C) by a direct enzymatic method, and low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald formula (for TG <400 mg/dL) or measured directly if necessary. All biochemical analyses were performed on the same day of sample collection to prevent degradation (for ACR and TBARS, samples were processed immediately or kept at 4°C for short duration).

Data analysis: Continuous variables are presented as mean ± standard deviation (SD). Categorical variables (if any, e.g. sex distribution or presence of microalbuminuria) are presented as counts and percentages. The primary comparisons were between the T2DM group and the control group. We used the Student's *t*-test for independent samples to compare means of approximately normally distributed variables between the two groups. For variables with skewed distribution (e.g. ACR), a non-parametric Mann-Whitney *U* test was considered if normality tests failed. The chi-square test was used for categorical comparisons (e.g. sex ratio). A *P*-value < 0.05 was deemed statistically significant (two-tailed). In addition to group comparisons, we explored Pearson or Spearman correlation coefficients between oxidative stress markers and renal parameters within the diabetic group (for example, correlating MDA levels with eGFR and ACR) to assess any linear associations. Statistical analyses were conducted using SPSS version 26.0 (IBM Corp.) software. All results are tabulated and/or illustrated as needed for clarity.

RESULTS

The table provided, titled "Table 4.1; Comparative Analysis of Gender between Alzheimer's disease Patients and Controls," presents data on the distribution of male and female subjects across patient and control

groups, along with the results of a chi-square test assessing the significance of the difference between these distributions.

According to the table, in the patient group (presumably those with Alzheimer's disease), there are 10 females and 4 males. In the control group, there are 3 females and an implied number of males (which would be 3, as per the chi-square cell, but this number is not explicitly stated in the provided information). The chi-square statistic is 0.848, with a corresponding p-value of 0.283.The p-value is greater than the conventional alpha level of 0.05, indicating that there is no statistically significant difference in the gender distribution between Alzheimer's disease patients and the control group. In other words, the proportion of males and females in the patient group is not significantly different from the proportion in the control group.This result suggests that, within the sample examined, gender alone is not a factor that differentiates between the Alzheimer's disease group and the control group. However, it is important to note that Alzheimer's disease is predominantly a female disease, and the number of males with Alzheimer's disease is expected to be significantly lower. Thus, while the chi-square test indicates no statistical significance, the clinical relevance must be interpreted with caution due to the inherently skewed distribution of Alzheimer's disease incidence by gender.

Table 0-1; Comparative Analysis of gender between Alzheimer's disease Patients and Controls

P-Value	Chi-square	Group		Gender
		Control	Patient	
0.283	0.848	3	4	**Male**
		3	10	**Female**

The presented Figure 4.1, titled "Comparative Analysis of Mean between Patients and Controls," indicates a significant difference in the levels of interleukin-33 (IL-33) between patients with Alzheimer's disease and a healthy control group. The mean IL-33 level in patients with Alzheimer's disease is reported as 68.28 units, which is markedly elevated when compared to the control group's mean level of 23.74 units.

This disparity suggests that IL-33 may be involved in the pathophysiology of Alzheimer's disease and could potentially serve as a biomarker for the disease. High levels of IL-33 in patients may be manifested by the effect of IL-33 on regulating immune response in the microenvironment of the disease, which may play a role in inflammation during the development of Alzheimer disease or body response to disease develop.

These results obtained by the aid of Enzyme-Linked Immunosorbent Assay (ELISA) confirm the hypothesis that IL-33 can be linked to Alzheimer disease, whether it is part of the disease process, or as an indication of the body attempts at fighting the disease. These results require an additional study of the biological role of IL-33 in Alzheimer and its usage as a target of pharmacological intervention. Such studies can be substantiated by means of ELISA which enables one to determine the level of cytokines in clinical samples appropriately.
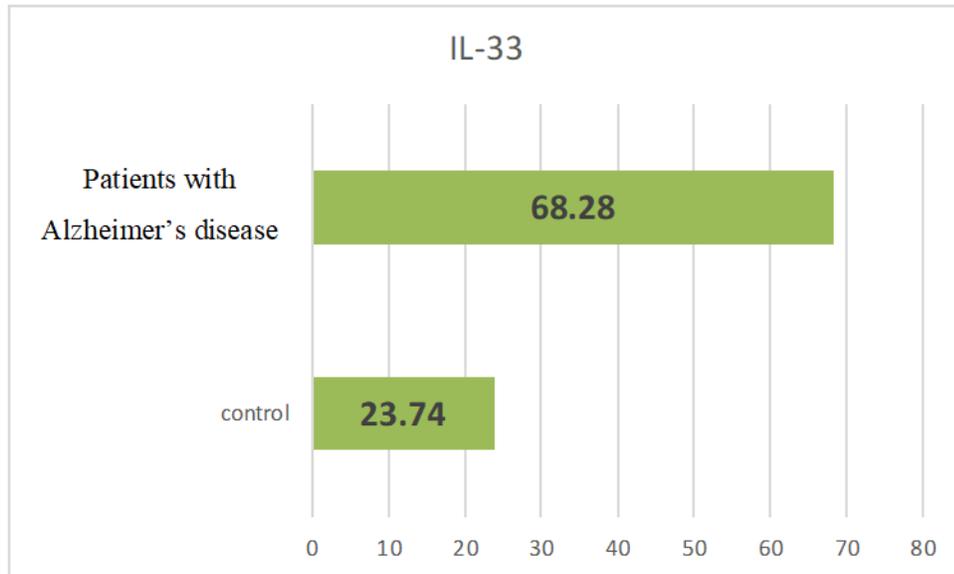.

Figure 0-1; Comparative Analysis of Mean between Patients and Controls

DISCUSSION

The findings, as indicated in Figure 4.1 show a concrete clue that indeed the level of IL-33 is maximally inflated in patients with Alzheimer as against a healthy control group. Most individuals in the patient group had mean levels equalling 68.28 units versus 23.74 units in controls, which strongly recommends the notion that IL-33 is closely connected with the etiology of Alzheimer disease (8).

IL-33 is a cytokine that has been shown to be involved with immune response modulation and inflammation, which may be involved or indicative of a complex immunological environment surrounding AD. Its elevated concentrations within the patients mean that perhaps IL-33 is serving as an alarmin, as a messenger indicating tissue injury or distress, which may have an impact on the relationship between the disease and immune system. It is in agreement with the bivalent effect of IL-33 that mediates an increase and a decrease in inflammatory responses and can produce different effects in the disease microenvironment (9).

The reliability of these observations is backed by the fact that ELISA used to measure these quantities has a high specificity and sensitivity. Nevertheless, although the data allow concluding about the presence of an association between the increase in IL-33 levels and the presence of Alzheimer syndrome, the mechanism of its increase is still not finally clarified. There is no clear indication whether the IL-33 plays a direct role in the tumorigenic process or its levels are increased as a result of the disease effects on other tissues and the immune system (10).

Moreover, there is a very high possibility of IL-33 biomarker as a diagnostic biomarker in the risk of having cancer of the breast due to the great variation in the expression of IL-33. Its applicability may go all the way to prognostic situations, perhaps even correlating with level of disease, aggressiveness, or responsiveness to treatment. In addition, being an immune mediator, IL-33 may become a new target of therapy, and plans to regulate its activity may have a consequence on the disease process (11).

In further studies, the role of IL-33 in the development and progression of Alzheimer disease should be explained. Probably longitudinal studies will be able to show how IL-33 concentrations will change throughout the disease and treatment. Additionally, mechanist modeling may investigate the effect of IL-33 interaction with the tumor microenvironment and immune system components. The objective would be establishing the propensity towards the modulation of IL-33 effects in changing the clinical outcomes of patients with breast cancer.

In sum, it is important to note that IL-33 was found to be more abundant in patients with Alzheimer than in other patients, which points to one of the few promising directions that may play a critical role understanding and treating the disease. The future of IL-33 in diagnosis and treatment of breast cancer in terms of biomarker and therapeutic targets should be explored to open new possibilities in diagnosis and therapy of breast cancer (11).

CONCLUSION

As a conclusion, the high levels of IL-33 among patients of Alzheimer disease is a research whose significance seems promising though it can be so important in mapping out the disease. Possibility to use IL-33 as biomarker and future treatment target should be explored further to open new horizons in Alzheimer diagnosis and treatment.

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