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Serum Antioxidant Profiling in Diabetes Mellitus Using a Novel Potassium Permanganate Redox Method

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Abstract

Background: Antioxidants have been increasingly recognized for their essential role in health and disease. Total serum antioxidant capacity (TSAC) has been estimated in diabetic patients. However, most commercially available kits that estimate both water and lipid soluble antioxidant components are often too expensive for routine patient care. In the present study, the feasibility of a low-cost semi-quantitative method for estimating the water-soluble components of TSAC in health and disease was explored.

Aim and Objectives: To perform a semi-quantitative estimation of the water-soluble antioxidant components of serum samples obtained from healthy individuals and patients with known cases of diabetes mellitus.

Methods: Serum samples were collected from healthy volunteers and clinically diagnosed diabetic patients. A known concentration of potassium permanganate ($KMnO_4$) solution was added to each sample. The decrease in absorbance at 540 nm was measured colorimetrically. The extent of reduction in $KMnO_4$ absorbance was considered reflective of the serum antioxidant status. Serial dilutions of vitamin C solution were subjected to the above test to generate a standard curve.

Results: Serum samples from diabetic patients exhibited significantly higher absorbance (0.4358 ± 0.03770) compared to healthy controls (0.2378 ± 0.03350), indicating reduced antioxidant capacity and elevated oxidative stress. The vitamin C dilutions demonstrated a linear plot, confirming the validity of the assay.

Conclusion: A simple $KMnO_4$-based semi-quantitative assay can effectively estimate water-soluble antioxidant components in serum. This inexpensive method may serve as a useful preliminary tool for assessing oxidative status in both healthy individuals and disease states.

Keywords: Antioxidant capacity, Diabetes mellitus, $KMnO_4$ assay, Oxidative stress, Redox status, Serum antioxidants

Introduction

Reactive oxygen species (ROS) are highly reactive molecules that function as important signalling mediators involved in host defence, gene regulation, and maintenance of cellular homeostasis.[1] Under physiological conditions, a balance exists between ROS generation and antioxidant defence mechanisms. Disturbance of this balance leads to oxidative stress, resulting in oxidative damage to lipids, proteins, and nucleic acids, ultimately contributing to cellular dysfunction and disease progression.[2] Oxidative stress has been implicated in the pathogenesis of several disorders, including diabetes mellitus, cardiovascular diseases, chronic kidney disease, neurodegenerative disorders, and vascular complications.[3]

To counteract oxidative damage, the human body possesses a complex antioxidant defence system comprising enzymatic and non-enzymatic antioxidants. Enzymatic antioxidants such as superoxide dismutase (SOD), catalase, and glutathione peroxidase catalyse the conversion of reactive oxygen intermediates into less toxic molecules.[4-5] Non-enzymatic antioxidants including ascorbic acid, uric acid, bilirubin, and reduced glutathione play an essential role in scavenging free radicals and maintaining redox balance.[5] Water-soluble antioxidants present in serum constitute an important component of systemic antioxidant defence and reflect the overall reducing potential of the body.[6-8]

Measurement of individual antioxidants alone may not accurately represent the total oxidative or antioxidant status of an individual.[6-9] Therefore, total antioxidant capacity (TAC) has emerged as a useful indicator of cumulative antioxidant activity and overall redox homeostasis.[10] Conventional TAC assays such as Ferric Reducing Antioxidant Power (FRAP), 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) assay (ABTS), 2,2-Diphenyl-1-picrylhydrazyl assay (DPPH), Oxygen Radical Absorbance Capacity assay (ORAC) methods are widely employed for antioxidant assessment; however, these techniques require specialised reagents, stringent reaction conditions, and advanced instrumentation.[7-11]

In recent years, simpler redox-based methods have been explored for assessing antioxidant capacity. One such approach involves the use of potassium permanganate ($KMnO_4$), a strong oxidising agent that undergoes reduction in the presence of antioxidant substances.[12] In this reaction, the purple potassium permanganate ($KMnO_4$) containing the permanganate ion (Mn^{7+}) is reduced to colourless manganese ion (Mn^{2+}) or brown manganese dioxide (MnO_2) depending on the reaction conditions. The extent of reduction is directly related to the antioxidant potential of the sample and can be quantified spectrophotometrically by measuring the decrease in absorbance at 540 nm.[12]

Diabetes mellitus is associated with chronic hyperglycaemia, increased generation of reactive oxygen species, glucose autoxidation, protein glycation, and impairment of antioxidant defence mechanisms, all of which contribute to enhanced oxidative stress.[14] Therefore, assessment of antioxidant status in diabetic individuals may provide valuable insight into disease-associated oxidative imbalance.

The present study was conducted to assess the total water-soluble antioxidant capacity of serum using a simple potassium permanganate ($KMnO_4$)-based redox titration method and to compare the antioxidant status of healthy individuals with that of patients with diabetes mellitus.

Rationale / Novelty

This study was based on the need for a simple, feasible, and cost-effective method to assess total serum antioxidant capacity (water-soluble component) using a $KMnO_4$-based assay.

Research Question

Can the $KMnO_4$-based assessment of the reducing potential of serum be used to reflect the antioxidant status in individuals in health and disease?

Objectives

1. To compare the $KMnO_4$-based estimation of total water-soluble antioxidant components in serum samples of healthy individuals and patients with diabetes mellitus in order to evaluate the potential of this simple redox titration method as an indicator of oxidative stress status and overall antioxidant capacity.

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2. To estimate the total water-soluble antioxidant capacity of serum samples from healthy individuals using the standardised $KMnO_4$ method.
3. To determine the total water-soluble antioxidant capacity of serum samples from patients with diabetes mellitus.

Methodology

A hospital-based case–control study was conducted over one month in the Department of General Medicine (Outpatient Department) and the Clinical Biochemistry Laboratory at Aarupadai Veedu Medical College and Hospital. The study included a total of 128 participants, comprising 64 cases and 64 controls. The sample size was calculated based on a comparison of means, considering an effect size of 0.5, a 5% level of significance, and 80% statistical power.

Study participants were selected by random sampling from the Department of General Medicine (Outpatient Department). Healthy volunteers aged 18–60 years were included as controls, while patients with diagnosed diabetes mellitus aged 18–60 years were included as cases. Individuals receiving antioxidant supplementation were excluded from the study. Demographic details and relevant clinical history were collected using a structured questionnaire. The aims and objectives of the study were explained to all participants, and written informed consent was obtained prior to enrolment. Appropriate blood samples were collected from all study participants under standard aseptic precautions and according to institutional guidelines.

The collected serum samples were analysed for total water-soluble antioxidant status using the potassium permanganate ($KMnO_4$)-based assay. All observations and laboratory findings were systematically recorded and documented for subsequent statistical analysis.

Reagents and Procedure

Random blood samples were collected under aseptic conditions and centrifuged to separate the serum. The reagents used for the assay included potassium permanganate ($KMnO_4$) solution at a concentration of 30 mg/dL. Standard vitamin C (ascorbic acid) solutions were prepared by serial dilution at concentrations of 20 mg/dL, 10 mg/dL, 5 mg/dL, 2.5 mg/dL, and 1.25 mg/dL, which were subsequently used to generate the standard calibration curve. For the assay, 500 µL of serum sample was mixed with 50 µL of potassium permanganate ($KMnO_4$) solution and 450 µL of distilled water, followed by incubation at room temperature for 2 minutes. The absorbance of the reaction mixture was then measured at 540 nm using a colorimeter. A reduction in absorbance was interpreted as an indicator of antioxidant activity, with the magnitude of the decrease being directly proportional to the antioxidant capacity of the serum sample

Statistical Analysis

Categorical variables were expressed as frequencies and percentages, whereas continuous variables were presented as mean ± standard deviation (SD). Comparisons between two groups were performed using the independent Student's *t*-test for normally distributed data and the Mann–Whitney U test for non-normally distributed data, as appropriate. For comparisons involving more than two groups, one-way analysis of variance (ANOVA) or the Kruskal–Wallis test was used, as applicable. A p-value < 0.05 was considered statistically significant. Statistical analyses were performed using SPSS Statistics version 29.

Results

The study population consisted of 128 participants, including 64 healthy controls and 64 patients with diabetes mellitus. The mean age was comparable between the control and case groups, thereby reducing the potential influence of age-related confounding factors on the study outcomes

The mean absorbance value in the control group was 0.2378 ± 0.03350, whereas the diabetic group exhibited a significantly higher mean absorbance value of 0.4358 ± 0.03770 (Table 1). In the $KMnO_4$ reduction assay, higher absorbance reflects a lower reduction of permanganate ions, indicating diminished antioxidant activity. Therefore, the elevated absorbance observed among patients with diabetes mellitus suggests a reduced total water-soluble antioxidant capacity and increased oxidative stress. In contrast, the lower absorbance values recorded in healthy controls indicate a more effective antioxidant defence system and a lower level of oxidative stress.

The mean difference in absorbance between the control and diabetic groups was −0.19797, with a

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narrow 95% confidence interval (−0.21045 to −0.18549), indicating a high degree of precision and reliability in the estimated effect. Statistical analysis revealed a highly significant difference between the two groups ($t = -31.402$, $p < 0.001$). A bar graph depicting the mean absorbance values of the control and diabetic groups is presented in Figure 1.

Discussion

The present study assessed the total water-soluble antioxidant capacity of serum using a potassium permanganate ($KMnO_4$)-based redox assay and compared the antioxidant status of healthy individuals with that of patients with diabetes mellitus. Oxidative stress is recognized as a key factor in the development and progression of diabetes mellitus, primarily due to chronic hyperglycaemia, which promotes excessive generation of reactive oxygen species (ROS) and compromises endogenous antioxidant defence mechanisms.[13]

The major finding of the present study was the significantly higher absorbance values observed among patients with diabetes mellitus compared to healthy controls. As the assay is based on the reduction of potassium permanganate ($KMnO_4$) by antioxidant compounds, an increase in absorbance indicates a lower degree of permanganate reduction and, consequently, diminished antioxidant activity. Thus, the elevated absorbance recorded in diabetic individuals suggests a reduction in total water-soluble antioxidant capacity, reflecting an increased burden of oxidative stress.

The highly significant difference observed between the diabetic and control groups ($t = -31.402$, $p < 0.001$) further supports the strong association between diabetes mellitus and oxidative imbalance. Chronic hyperglycaemia is known to enhance oxidative stress through several mechanisms, including glucose autoxidation, formation of advanced glycation end products (AGEs), activation of the polyol pathway, and increased mitochondrial generation of reactive oxygen species (ROS).[13] Collectively, these processes lead to excessive free radical production, depletion of endogenous antioxidant defences, and subsequent cellular and tissue damage.

The findings of the present study are consistent with previous reports demonstrating reduced antioxidant capacity in patients with diabetes mellitus.[6–9] The observed decrease in antioxidant status among diabetic individuals further supports the role of oxidative stress in the pathogenesis and progression of the disease.

The potassium permanganate ($KMnO_4$)-based assay employed in this study offers several practical advantages over conventional total antioxidant capacity (TAC) assays such as Ferric Reducing Antioxidant Power (FRAP), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and Oxygen Radical Absorbance Capacity (ORAC). The assay is simple, rapid, cost-effective, and requires minimal instrumentation, making it suitable for routine laboratory applications, particularly in resource-limited settings.

However, certain limitations should be considered. The present method primarily evaluates water-soluble antioxidant components and does not account for lipid-soluble antioxidants, such as vitamin E and carotenoids, which also contribute significantly to the overall antioxidant defence system. Therefore, the measured antioxidant capacity may not fully reflect the total antioxidant status of an individual.

Conclusion

The present study demonstrated a significant reduction in total water-soluble antioxidant capacity among patients with diabetes mellitus compared to healthy controls, highlighting the increased oxidative stress associated with the disease. The potassium permanganate ($KMnO_4$)-based redox assay was found to be a simple, reliable, rapid, and cost-effective method for the assessment of serum antioxidant status. Given its ease of performance and minimal instrumentation requirements, this assay may serve as a valuable tool for the routine evaluation of oxidative stress in clinical laboratories, particularly in resource-constrained settings. Future research involving larger populations and detailed assessment of both water-soluble and lipid-soluble antioxidants is required to validate and strengthen these observations..

Previous Presentation

This study was presented at DECCON (Decode the Error Code) conducted by the Department of Biochemistry, Sree Balaji Medical College & Hospital from 05.11.2025 to 07.11.2025.

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Ethical Approval

The Institutional Ethical Committee of Aarupadai Veedu Medical College and Hospital approved the study protocol (IEC No. AV/IHEC/February 2026/10). Written informed consent was obtained from all participants before sample collection.

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Table 1: Comparison of Absorbance Levels Between Control and Case Groups

Parameter	Control Group (n = 64)	Case Group (n = 64)	Mean Difference	p-value
Absorbance (Mean ± SD)	0.2378 ± 0.03350	0.4358 ± 0.03770	−0.19797	<0.001*

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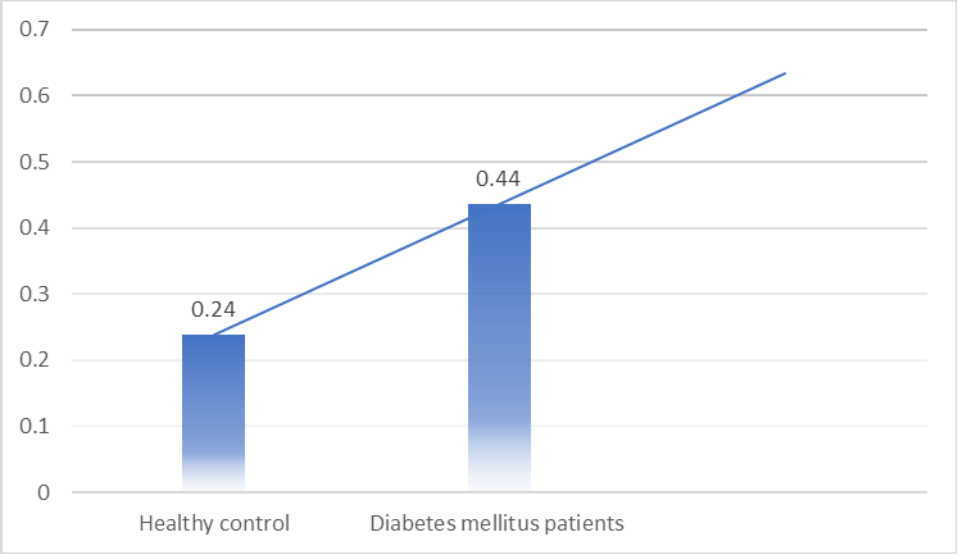
Figure 1: Comparison of Mean Absorbance Values Between Healthy Controls and Diabetic Patients ($KMnO_4$ Reduction Assay at 540 nm)



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