Article

Assessing Oxidative Stress and Antioxidant Status in Hypertensive Patients in the Governorate of Basrah, Iraq

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ABSTRACT

Hypertension is a serious public health issue not just in Iraq but throughout the world. This work sought to assess the effect of oxidative stress by assessing levels of protein carbonyl and antioxidant enzymes in hypertension patients compared to a healthy control group in Basra, Iraq. Reactive oxygen species (ROS) are important in oxidative stress. The study included 100 hypertensive patients (44 males and 56 females) and 50 healthy individuals (25 males and 25 females) aged between 35 and 70 years. Venous blood samples were collected from all participants. Known by another name, the silent killer, hypertension is a serious public health issue not just in Iraq but throughout the world. This work sought to assess the effect of oxidative stress by assessing levels of protein carbonyl and antioxidant enzymes in hypertension patients compared to a healthy control group in Basra, Iraq. Reactive oxygen species (ROS) are important in oxidative stress. All studied antioxidant enzymes showed high discriminatory ability between hypertensive patients and healthy individuals through ROC analysis. The study shows that oxidative stress is a major factor in the development of high blood pressure in people in Basra, Iraq. It also shows the connection between lower antioxidant enzyme activity and higher oxidative stress. The study suggests the use of antioxidants as a successful tool for disease detection and considers them key chemical indicators of hypertension.

Key words: Hypertension, oxidative stress, antioxidant enzymes, SOD, CAT, GSTs, GPX3, protein carbonyl (PC), ROC analysis.

Introduction

Oxidative stress is a condition characterized by an imbalance between the production of reactive oxygen species (ROS) and the body's capacity to neutralize them [1]. (ROS) are made naturally by the body as a result of metabolism. They can also be made by things in the environment, like pollution, radiation, and smoking. ROS can hurt cells and organs by mixing with the DNA, proteins, and fats of those cells. Many health issues, like cancer, heart disease, and brain diseases [2], can be caused by this damage. Over time, more and more data has shown that oxidative stress can lead to high blood pressure. Researchers have found that people with high blood pressure have more reactive oxygen species (ROS) in their blood than people with normal blood pressure [3, 4]. Oxidative stress can raise blood pressure through a number of different pathways. One mechanism is that ROS can inhibit the production of nitric oxide (NO), a molecule that relaxes blood vessels and lowers blood pressure. [5] Nitric oxide (NO) is produced by endothelial cells that line the inner walls of blood vessels. ROS can interact with NO and deactivate it, leading to vessel constriction and increased blood pressure [6, 7]. Another way oxidative stress can contribute to high blood pressure is by promoting inflammation. Inflammation can damage blood vessels and lead to arterial narrowing, which can increase blood pressure [8, 9]. Finally, oxidative stress can contribute to the development of atherosclerosis, a condition in which plaques accumulate in the arteries. Plaque buildup can narrow arteries and reduce blood flow, leading to increased blood pressure [10, 11]. The generation of free radicals leads to the peroxidation of fats. Subsequently, lipid peroxides can decompose to form aldehydes. Aldehydes are characterized by containing a carbonyl group and having the ability to react with proteins, leading to the formation of carbonylated proteins [12, 13]. These chemicals show signs of oxidative protein damage and can change the structure and function of proteins, which can damage cells and cause many illnesses [14, 16]. Among the most sensitive indicators of chronic oxidative stress are carbonylated proteins. This phenomenon arises from the oxidation of amino acids within protein structures. The accumulation of carbonylated proteins over time provides evidence of long-term oxidative stress. Therefore, the utilization of carbonylated proteins is beneficial in the medical diagnosis of diseases characterized by chronic oxidative stress, such as cardiovascular diseases, high blood pressure, and cancer [17, 19]. Free radicals interact with proteins containing thiol groups because they view them as a source of electrons. When the thiol group interacts with free radicals, the unpaired electrons are donated to the free radicals, thereby

removing electrons from them. This stabilizes the free radicals but leaves the thiol group in an oxidized state. Oxidation of the thiol group can lead to a variety of changes in protein structure, including the formation of disulfide bonds between different thiol groups, alterations in the three-dimensional shape of the protein, and loss of protein function [20, 22]. The aims of study were estimation of enzymatic antioxidant activities (SOD, CAT, GSTs, GPX3) in hypertensive patients and comparison with healthy individuals, evaluation of oxidative stress intensity by studying PC levels in hypertensive patients and comparison with healthy individuals, analysis of the correlation between the studied variables, and testing the effectiveness of antioxidants in predicting the course of hypertension and considering them as a useful diagnostic tool in patients.

Materials and methods

Participants

A case-control study recruited 100 hypertensive patients aged 35–70 years (44 males, 56 females) without heart, kidney, or diabetes history, confirmed at the Hypertension and Diabetes Unit in Al-Hartha District, Southern Iraq. The control group comprised 50 individuals with normal blood pressure, aged 35–70 years (25 males and 25 females), free from apparent diseases and a negative family history of heart disease, diabetes, and hypertension, with normal blood pressure after three measurements. The age categories of the participants were 35–45, 46–56, and over 57.

Blood collection and processing

Five milliliters of blood were drawn using a syringe from both patients and healthy people, then put into test tubes containing gel and centrifuged for ten minutes at 3000 rpm to separate the serum. After that, the serum was divided into microtubes and kept in a deep freezer until it could be examined.

Biochemical measurements

The amounts of enzymatic antioxidants, which are made up of SOD, CAT, GSTs, and GPX3, were checked in the blood serum of both sick and healthy people. The enzyme-linked immunosorbent test (ELISA) method was also used to measure the amount of PC, which is another sign of oxidative stress in the body [23, 24].

Ethical approval and study design:

There was approval for the study from the Ethics Committee of the University of Basrah's College of Education for Pure Sciences' Department of Chemistry on March 11, 2021. The approval number was 3335/18/3. In line with the Helsinki Declaration and other relevant ethical standards, the study was carried out. Everyone who took part in this study gave verbal approval.

Statistical analysis

All of the data were statistically examined using the Microsoft Excel 2021 graphic and SPSS v.28 programs. Statistics of normal distribution for group differences and mean ± standard error is the way that data are reported. Comparison of continuous variables between groups using independent T-tests. Every time, a one-way ANOVA with Tukey's post hoc was used to do multiple group comparisons. The chi-square test was used for categorical variable analysis. For the diagnosis of the independent variables, correlation coefficient analysis was followed by ROC curve analysis and AUC. There was a $p<0.05$ significance of differences.

RESULTS

The results in Table (1) demonstrated the general studied characteristics in hypertension patients and control groups.

Table 1 Distribution of demographic variables in hypertension patients and control groups

Variables	Categories	Patients	Control	Chi-Square	p-value
Age (year) mean± SE		53.84±0.84	51.26±1.17		0.078
Age groups	**35_45**	25 25.0%	16 32.0%	5.121	0.077
	46_56	30 30.0%	21 42.0%		
	> 57	45 45.0%	13 26.0%		
Gender	**Female**	56 56.0%	25 50.0%	0.483	0.487
	Male	44 44.0%	25 50.0%		
BMI (kg/m2) mean± SE		30.74±0.55	29.14±0.73		0.087
BMI groups	**Normal weight**	14 14.0%	12 24.0%	4.853	0.088
	Overweight	34 34.0%	21 42.0%		
	Obesity	52 52.0%	17 34.0%		
Family history	**Genetic**	40 40.0%	0 0.0%	4.00 [a]	0.046*
	Non genetic	60 60.0%	0 0.0%		
Illness period mean± SE		5.67±0.36	0		
Period	**1_5 year**	55	0	22.340 [a]	<0.001*

groups		55.0%	0.0%		
	6_10 year	27 27.0%	0 0.0%		
	11_15 year	18 18.0%	0 0.0%		
Food-adherence	**Regular**	44 44.0%	0 0.0%	1.440 [a]	0.230
	Irregular	56 56.0%	0 0.0%		
Smoking	**No**	36 36.0%	50 100.0%	7.840 [a]	0.005
	Yes	64 64.0%	0 0.0%		

*Significant differences at p-value <0.05. all data expressed as frequencies and percentage. Chi-Square test.
a: comparison between groups of patients only.

Serum Antioxidant levels in hypertension patients and control groups

The statistical analysis in Table 2 shows the hypertension patients have a mean SOD level of 4.68 ± 0.18 ng/ml and a mean CAT level of 2.62 ± 0.11 ng/ml, significantly ($p < 0.001$) lower than the control group's mean of 27.99 ± 0.59 and 11.09 ± 0.75, respectively. At similar findings, means GST levels of 14.83 ± 0.87 ng/ml and GPX3 levels of 41.04 ± 0.99 ng/ml were significantly lower than the control group's means of 55.01 ± 3.13 and 98.05 ± 1.22 ng/ml ($p < 0.001$), respectively. In contrast, a significantly higher mean PC level of 62.1 ± 1.46 ng/ml was observed in hypertension patients when compared with the control group of 29.02 ± 1.95 ng/ml ($p < 0.001$).

Table 2. Comparison of serum Antioxidant levels in hypertension patients with control groups

Variables	Patients n=100	Control n=50	p-value
SOD (ng/ml)	4.68±0.18	27.99±0.59	0.0001*
CAT (ng/ml))	2.62±0.11	11.09±0.75	0.0001*
GSTs (ng/ml)	14.83±0.87	55.01±3.13	0.0001*
GPX3 (ng/ml)	41.04±0.99	98.05±1.22	0.0001*
PC (ng/ml)	62.1±1.46	29.02±1.95	0.0001*

Correlation of Between Antioxidants in Hypertension Patients

SOD had positive correlations with CAT ($r = 0.547$, $p < 0.01$), figure (1), GSTs ($r = 0.618$, $p < 0.01$), and GPX3 ($r = 0.346$, $p < 0.01$), figures (2) and (3). Nevertheless, it showed a negative correlation ($r = -0.657$, $p < 0.01$) with PC (Figure 4). Figures (5) and (6) demonstrate the positive correlation of the CAT results with GSTs ($r = 0.539$, $p < 0.01$) and GPX3 ($r = 0.344$, $p < 0.01$). But

PC was shown to be inversely linked ($r = -0.483$, $p < 0.01$); see figure 7. Significantly positive association ($r = 0.307$, $p < 0.01$) between GSTs and GPX3 and negatively correlated with PC ($r = -0.648$, $p < 0.01$), figure (9). Moreover, strongly significantly negatively correlated the results of GPX3 with PC ($r = -0.391$, $p < 0.01$) (Figure 10).

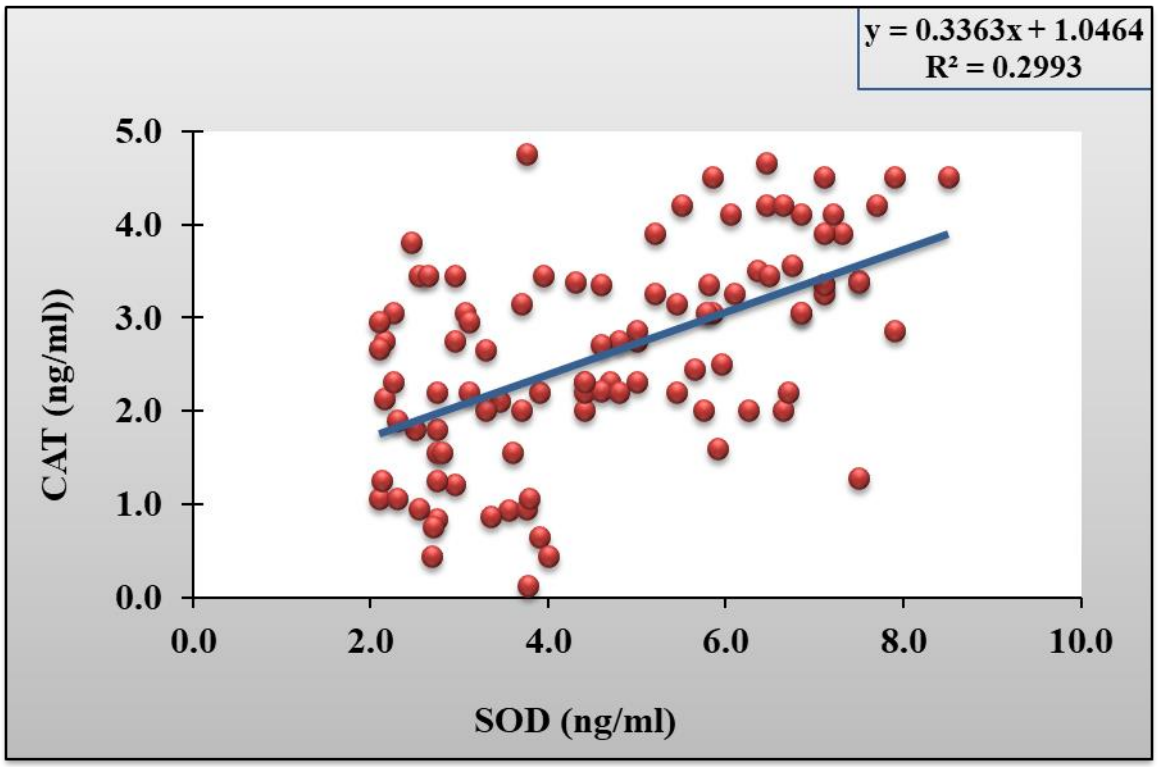

Figure 1. Correlation of SOD with CAT in hypertension patients. *Significant differences at p-value **<0.01, p*<0.5*

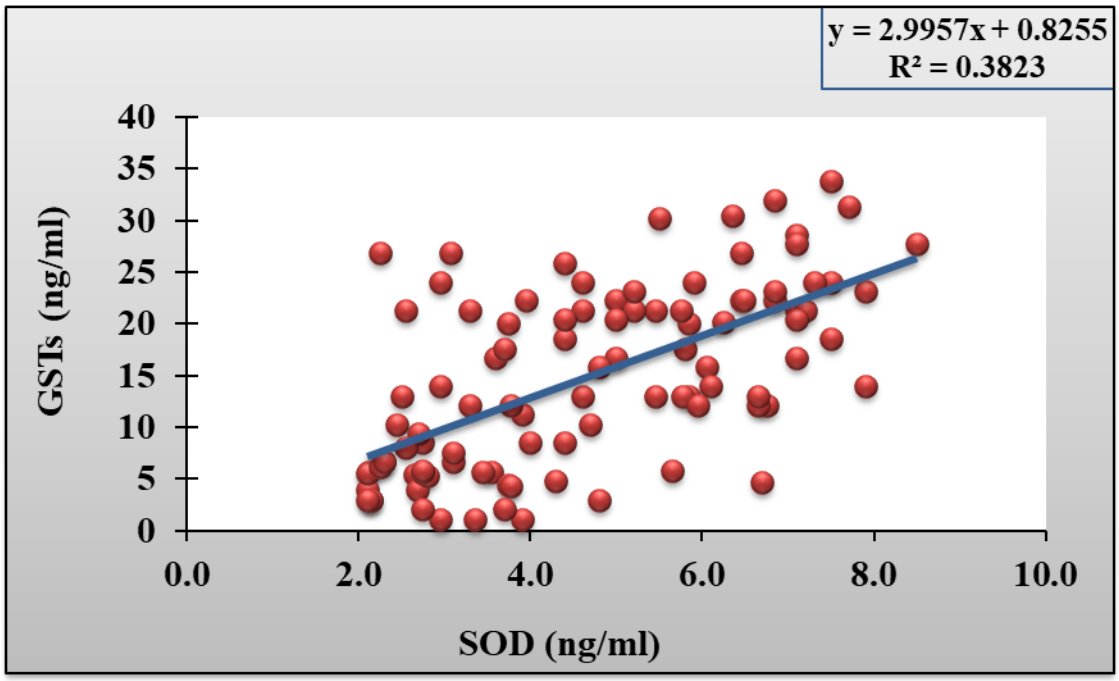

Figure 2. Correlation of GSTs with SOD in hypertension patients. Significant differences at p-value **<0.01, p*<0.5.

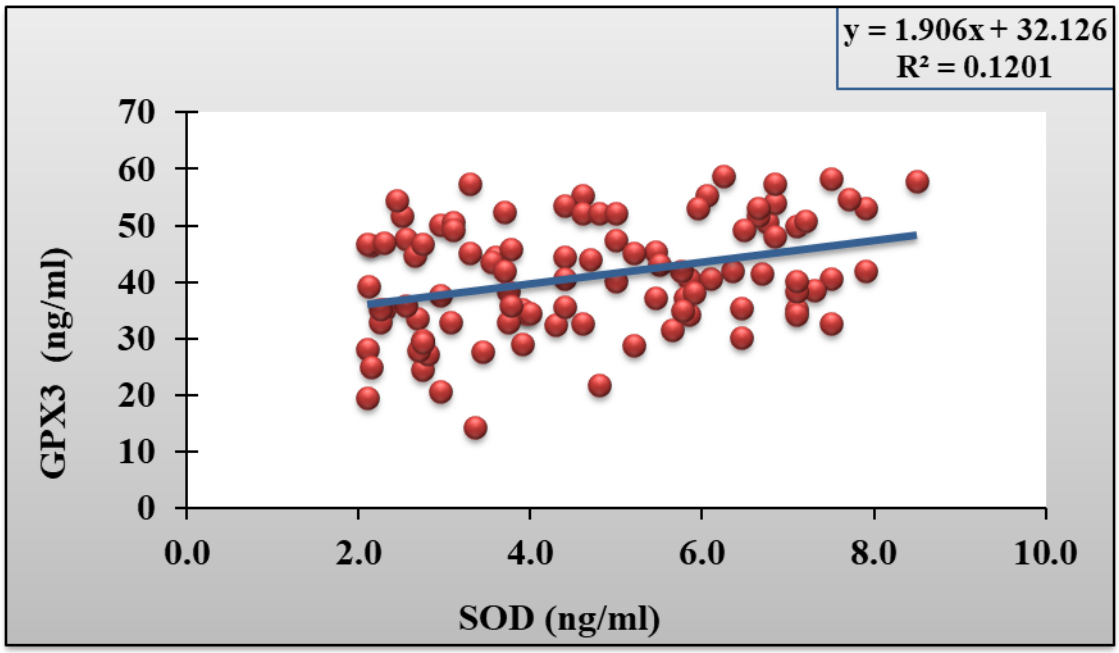

Figure 3. Correlation of GPX3 with SOD in hypertension patients. *Significant differences at p-value **<0.01, p*<0.5.*

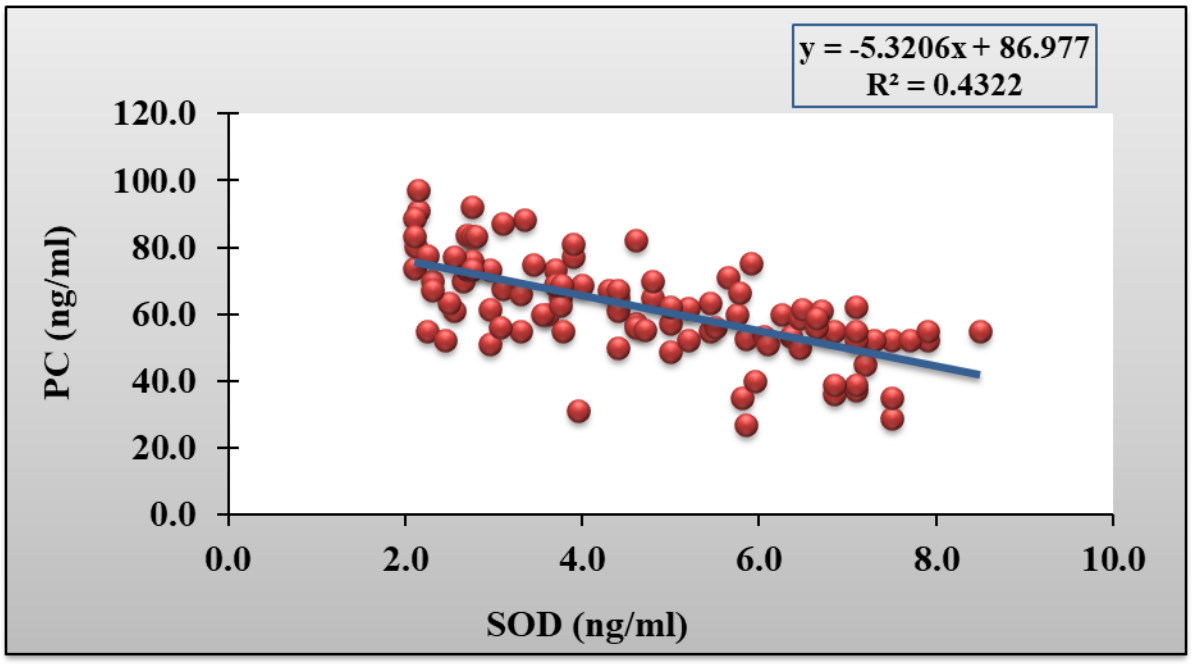

Figure 4. Correlation of PC with SOD in hypertension patients. *Significant differences at p-value **<0.01, p*<0.5.*

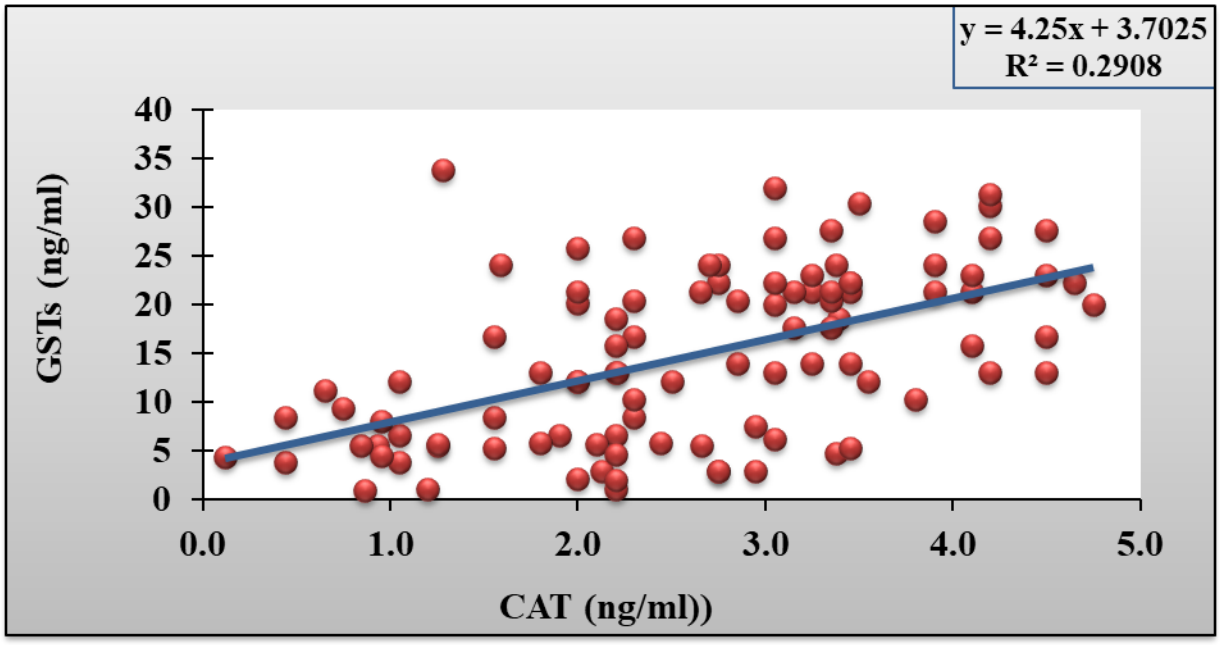

Figure 5. Correlation of GSTs with CAT in hypertension patients. *Significant differences at p-value **<0.01, p*<0.5.*

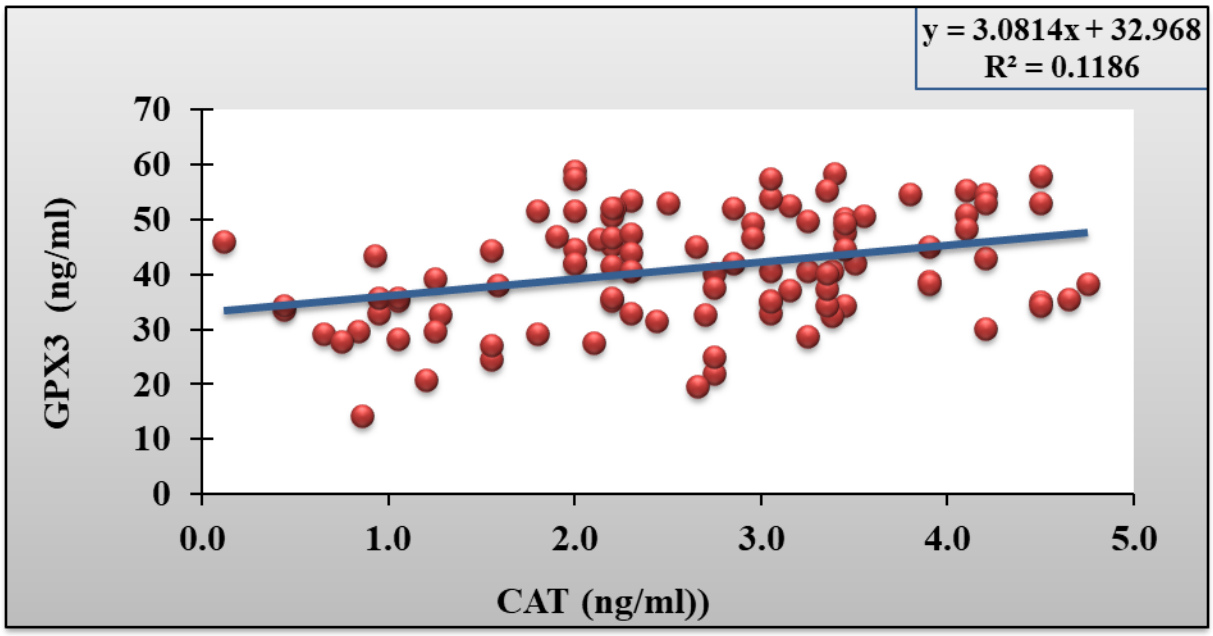

Figure 6. Correlation of GPX3 with CAT in hypertension patients. *Significant differences at p-value **<0.01, p*<0.5.*

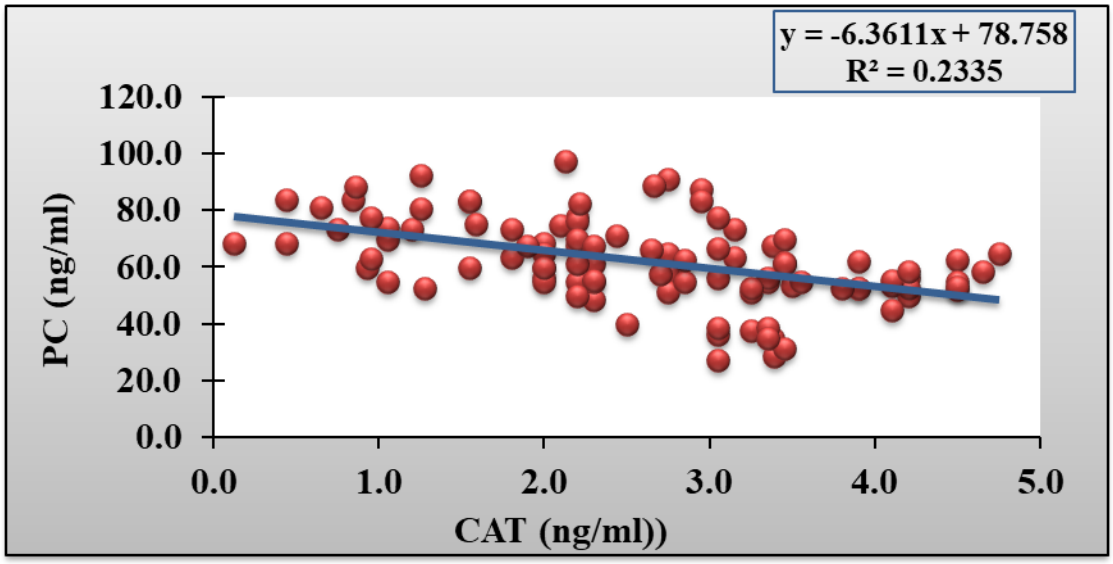

Figure 7. Correlation of PC with CAT in hypertension patients. *Significant differences at p-value **<0.01, p*<0.5.*

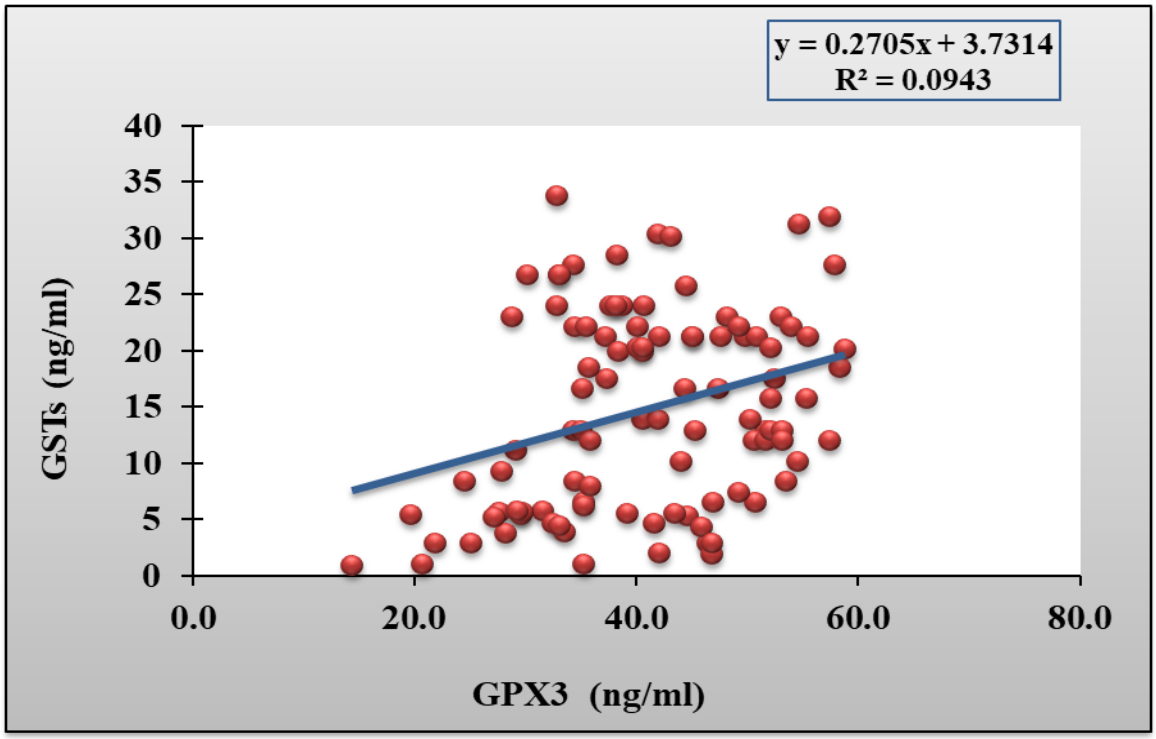

Figure 8. Correlation of GPX3 with GSTs in hypertension patients. *Significant differences at p-value **<0.01, p*<0.5*

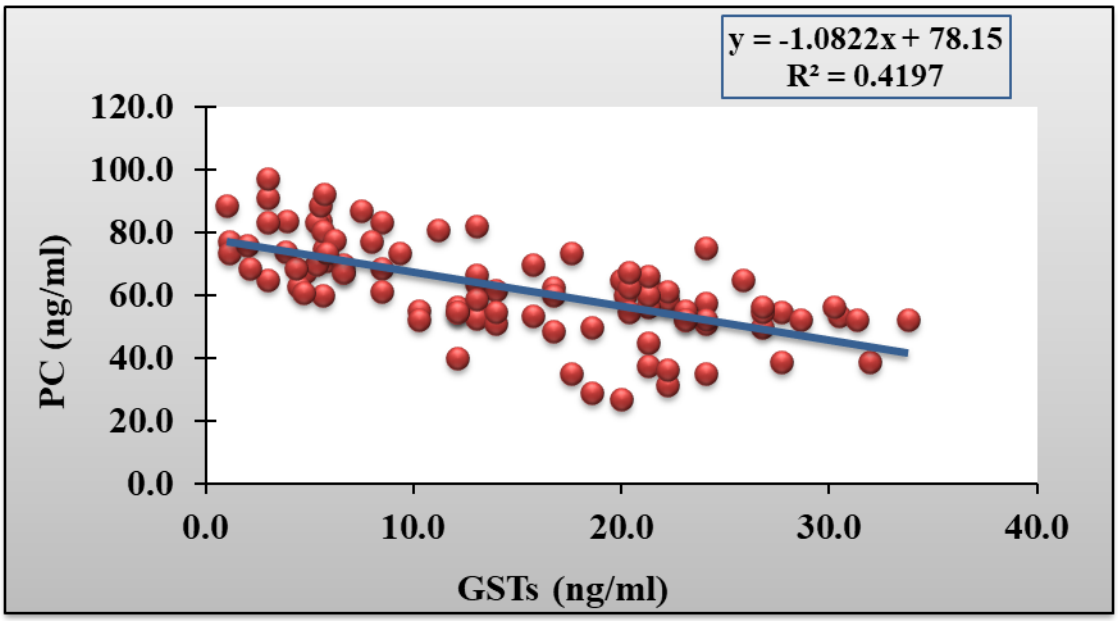

Figure 9. Correlation of PC with GSTs in hypertension patients. *Significant differences at p-value **<0.01, p*<0.5*

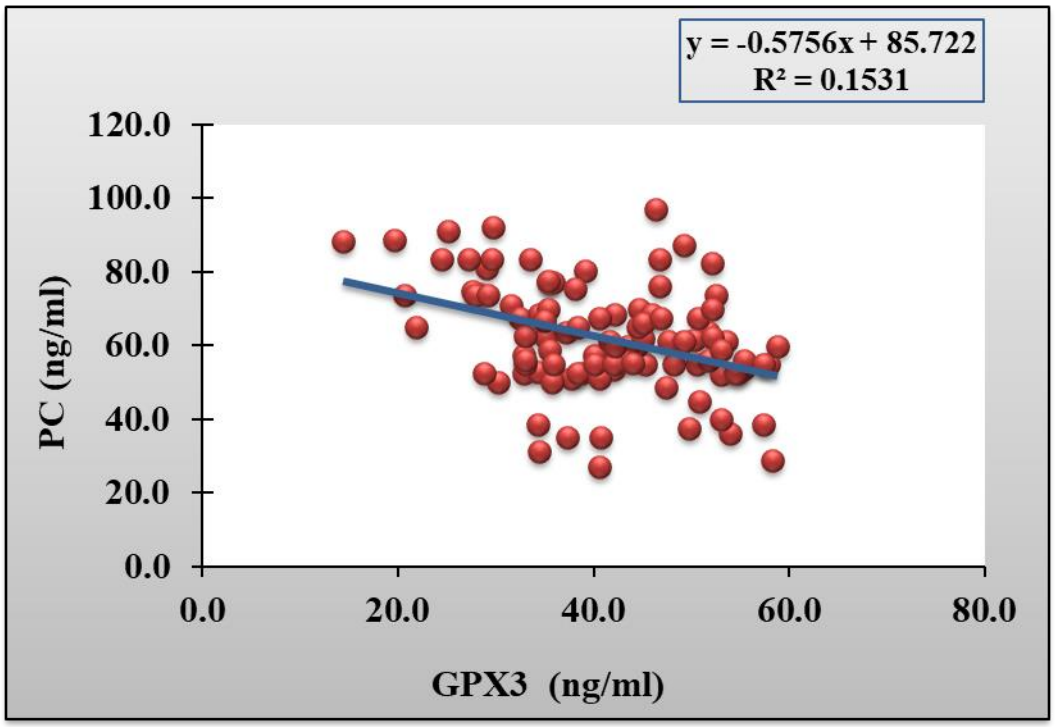

Figure 10. Correlation of PC with GPX3 in hypertension patients. *Significant differences at p-value **<0.01, p*<0.5.*

Analysis of Receiver Operation Characteristics for Predict risk factors to hypertension.

Table 3 and Figure 1 report the ROC analysis to assess the performance of a predictive model in distinguishing between patients and controls for various antioxidants as biomarkers in predicting risk factors for hypertension. SOD and GPX3 exhibit perfect predictive ability for hypertension risk factors. They demonstrate perfect discriminatory power with values of AUC: 1.00, 95% CI: [1.00, 1.00], and the cut-off: less than 4.7750 ng/ml, with sensitivity-specificity: 1.00–1. 00, and AUC: 1.00, 95% CI: [1.00, 1.00], and the cut-off: less than 69.6100 ng/ml, with sensitivity-specificity: 1.00–0.24. Therefore, CAT of AUC: 0.983, 95% CI: [0.957, 1.009], cutoff: 4.5250 ng/ml, sensitivity-specificity: 0.980-0.96, more than GST of AUC: 0.951, 95% CI: [0.915, 0.987], cutoff: 30.90 ng/ml, sensitivity-specificity: 0.970-0.84. Also, it shows a strong predictive ability of PC with an AUC of 0.951, a 95% CI of [0.921, 0.981], and a cut-off of more than 51.675 ng/ml, with a sensitivity-specificity of 0.840-0.60 (Figure 12).

Table 3. Analysis of Receiver Operation Characteristics for Predict antioxidant as risk factors to hypertension

Antioxidant	AUC	p-value	95% CI		Cut-off	Sensitivity	Specificity
			Lower Bound	Upper Bound			
SOD (ng/ml)	1.000	0.0001	1.000	1.000	<14.7750	1.000	1.00
CAT (ng/ml))	0.983	0.0001	0.957	1.009	<4.5250	0.980	0.96
GSTs (ng/ml)	0.951	0.0001	0.915	0.987	<30.9000	0.970	0.84
GPX3 (ng/ml)	1.000	0.0001	1.000	1.000	<69.6100	1.000	0.23
PC (ng/ml)	0.951	0.0001	0.921	0.981	>51.6750	0.840	0.60

AUC: area under curve. CI: 95% Confidence Interval

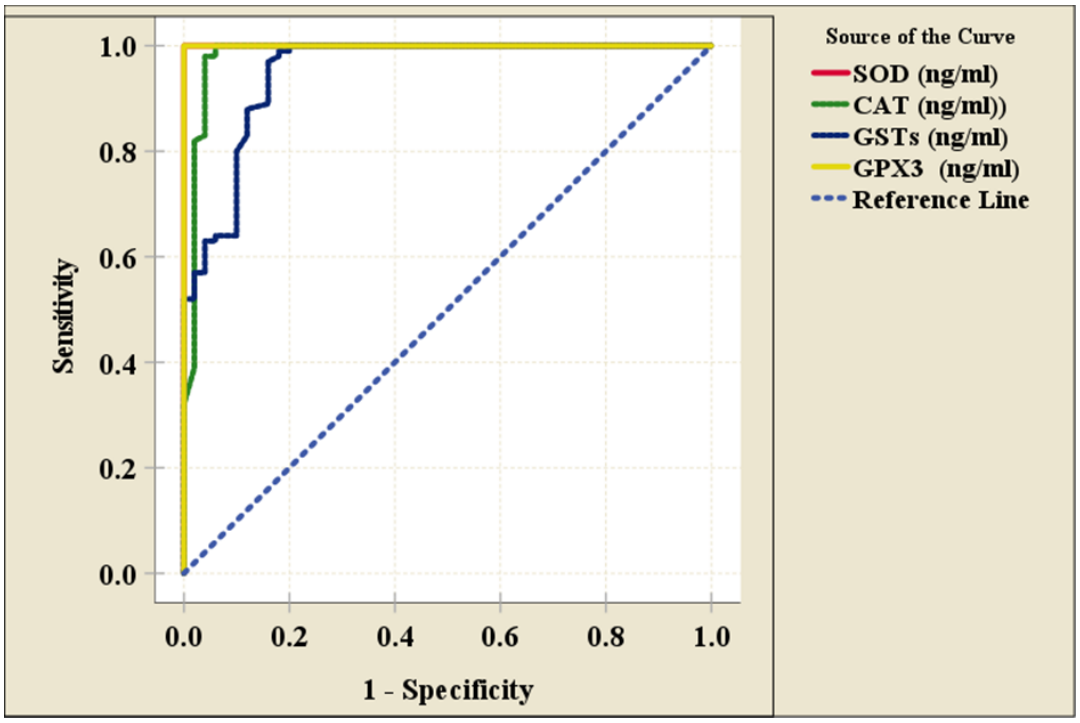

Figure 11. ROC Predict SOD, CAT, GSTs, AND GPXs Antioxidants in the hypertension patients. *Significant differences at p-value **<0.01. a: The category references are control group. 95% CI: Confidence Interval for AUC (area under the curve).*

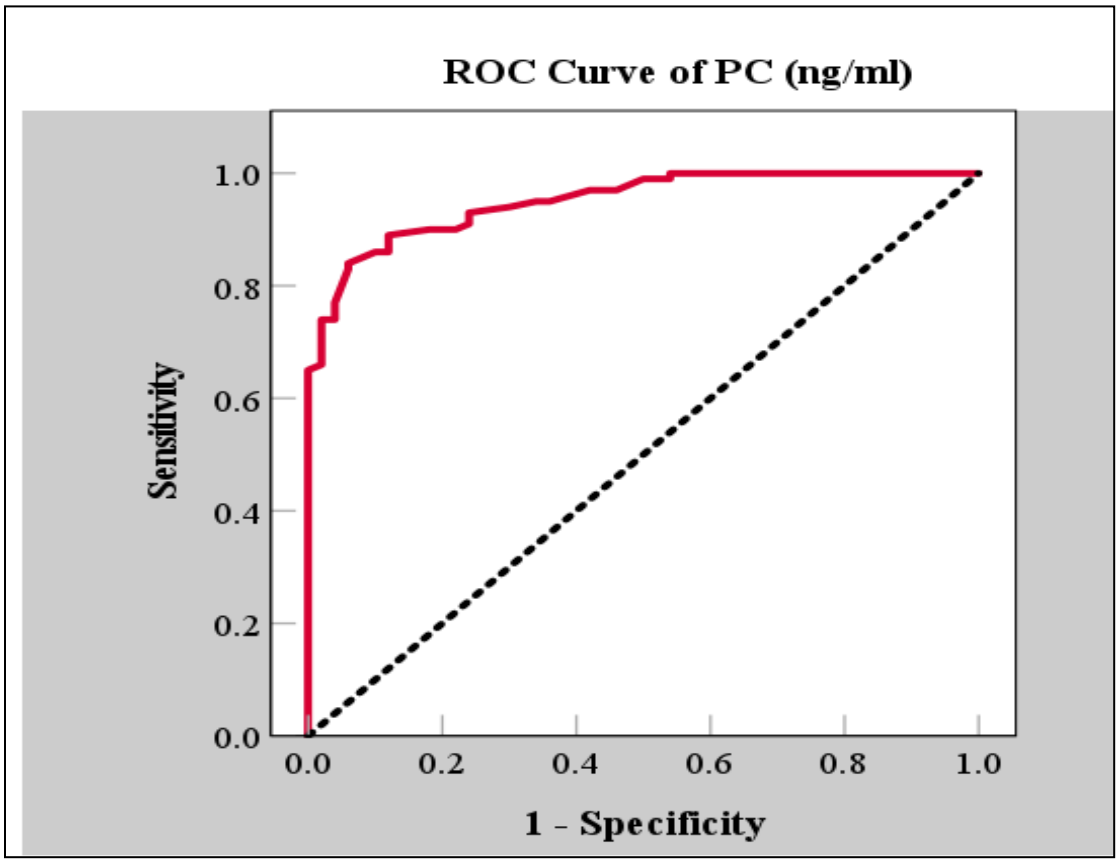

Figure 12. ROC Predict PC Antioxidant in the hypertension patients. Significant differences at p-value **<0.01. a: The category references are control group. 95% CI: Confidence Interval for AUC (area under the curve).

Discussion

Table 2 shows an important result from the study: all antioxidant enzymes studied are much less active in people with high blood pressure compared to the control group. This fits with a number of earlier studies [25, 26]. Superoxide radicals (O2) contribute significantly to oxidative stress and are a major source of reactive nitrogen species and other reactive oxygen species (ROS). It is because these radicals react with nitric oxide in blood vessels that nitric oxide is less bioavailable. Nitric oxide is an important part of vasodilation. The enzyme superoxide dismutase (SOD) is very important because it breaks down superoxide radicals into less dangerous substances. This keeps cells safe from oxidative damage and controls how other antioxidant enzymes work [27]. This explains the reduced levels observed in patients. Studies have shown that different types of SOD enzymes have beneficial antioxidant effects in various conditions, including eye and cardiovascular diseases, as well as metabolic disorders such as diabetes and obesity [28, 29]. The study also showed a decrease in catalase (CAT) enzyme levels in patients. Catalase enzyme levels in hypertensive patients have been extensively studied in the literature. Research has consistently

shown a significant decrease in catalase levels in hypertensive individuals compared to healthy controls, indicating a potential link between hypertension and altered antioxidant enzyme activity [30, 31]. Catalase is very important for lowering oxidative stress because it turns hydrogen peroxide into water and oxygen [32]. Consistent with previous findings, our study observed a significant decrease in glutathione-S-transferase (GST) enzyme levels in hypertensive individuals compared to healthy controls. This reduction in enzyme activity underscores the importance of maintaining robust antioxidant systems to combat oxidative stress in hypertensive patients [33]. The observed GST enzyme deficiency aligns with other studies suggesting that impaired GST activity may contribute to the development and elevation of blood pressure [34]. Finally, the study found a significant decrease in GPx3 enzyme levels. GPx3 is an antioxidant enzyme essential for maintaining cardiovascular health. It reduces oxidative stress and inflammation, both of which contribute to hypertension and other heart diseases [35]. Studies suggest that low GPx3 levels or impaired activity are associated with hypertension and its complications [36]. Apart from the reduced antioxidant enzyme levels, the study revealed that the protein carbonyl levels of hypertension patients were significantly higher than those of the control group. One explanation for the higher protein carbonyl levels in hypertensive people is the body's heightened oxidative stress [37]. This oxidative stress is likely to arise from factors such as increased pressure on blood vessel walls and increased mechanical stimulation, which are hallmarks of hypertension [38, 39]. The association analysis results (Figures 1–10) show how complexly antioxidant enzymes work together. Each enzyme protects cells from oxidative damage by neutralizing reactive oxygen species (ROS) and working with the others to do their job [40–43]. In addition, some enzymes, like glutathione S-transferases (GSTs), help the body get rid of toxins by attaching them to glutathione. This makes cells stronger and lowers the risk of problems related to high blood pressure [44]. Furthermore, the negative correlations between protein carbonyl levels and antioxidant enzymes suggest a protective role for these enzymes against oxidative stress. These inverse relationships imply the existence of regulatory mechanisms governing the interplay between antioxidant enzymes and protein carbonylation levels, wherein elevated protein carbonylation stimulates antioxidant enzymes to enhance cellular defenses against oxidative stress [45, 46]. Highlighting the importance of a well-coordinated antioxidant defense system in cell health and disease prevention. The reported values of the area under the curve (AUC) and the specified cut-off points in Table (3) and Figure (11) indicate that these enzymes, particularly SOD, GPX3, and CAT, are vital biomarkers that hold promising potential in assessing the risk factors associated with hypertension [47, 48].

Conclusion

High blood pressure is a chronic metabolic disorder. Oxidative stress plays an important role in its development. Therefore, the current study was conducted to assess levels of antioxidant enzymes, revealing a decrease in antioxidant enzyme levels and an increase in protein carbonyl levels in patients. This emphasizes the connection between high blood pressure, elevated oxidative stress, and reduced antioxidant enzyme activity. The study also proposes the possible application of antioxidant enzymes as important biochemical markers for hypertension and as a diagnostic tool.

Declarations

Ethical approval

This study was performed according to the ethical rules for medical research involving human participants of the Declaration of Helsinki (1964). Ethical approval was received from the ethical and research committee of the University of Basrah - College of Education for Pure Sciences - Department of Chemistry, with the number (3335/18/3) on 3/11/2021. Informed consent was obtained from all caregivers of participated.

-Availability of data and material The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

-Competing interests The author declare that they have no competing interests

-Funding No funds were received to fulfil this work.

- **Author contribution** The author was contributed in conceptualized the research, collected data, participated in data analysis and write-up, editing and review.

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