ISSN: 0067-2904

Association of Superoxide Dismutase with Ticagrelor Treatment in Patients with Peripheral Arterial Disease

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Received**:** 9/2/2024 Accepted: 1**3**/**6**/2024 Published: 30/5/2025

Abstract

Peripheral artery disease (PAD) is associated with increased oxidative stress and impaired endothelial function. Ticagrelor treatment improves antioxidant properties in addition to its antiplatelet effects. This study investigated the impact of Ticagrelor treatment on serum superoxide dismutase (SOD) levels and other biochemical parameters in PAD patients. It also evaluated the potential diagnostic accuracy and clinical utility of specific biomarkers based on receiver operating characteristic (ROC) analysis. Seventy individuals were categorized into healthy control (n=40), baseline PAD patients not on Ticagrelor (B-PAD, n=30), and same PAD patients after treated with Ticagrelor (A-PAD, n=30). Parameters measured included SOD concentration/activity, oxidized protein levels, serum trace elements, and other biochemical parameters. PAD patients showed elevated oxidized protein level and reduced SOD concentration and activity versus control group. Ticagrelor improved antioxidant status in PAD via SOD upregulation and reduced protein oxidation. Parameters of oxidative stress/antioxidants exhibited potential for PAD screening, diagnosis and longitudinal monitoring of treatment response using the diagnostic metrics derived from ROC analysis.

Keywords: Cu/Zn-SOD, Mn-SOD, oxidized protein, peripheral arterial disease, Ticagrelor.

ارتباط السوبر اوكسيد دسميوتيز وعلاج التيكاجريلور في المرضى المصابين بالشرايين المحيطية

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الخلاصة

يرتبط مرض الشريان المحيطي بزيادة الإجهاد التأكسدي وضعف وظيفة بطانة الأوعية الدموية. يعمل علاج التيكاجريلور على تحسين خصائص مضادات الأكسدة بالإضافة إلى تأثيراته المضادة للصفائح الدموية. بحثت هذه الدراسة في تأثير علاج تيكاجريلور على مستويات السوبر اوكسيد دسميوتيز في مصل الدم والمعلمات البيوكيميائية الأخرى لدى مرضى الشريان المحيطي. كما قيمت هذه الدراسة الدقة التشخيصية المحتملة والفائدة السريرية لمؤشرات حيوية محددة بناءً على تحليل الخصائص العملياتية للمستقبِل. تم تصنيف سبعين فردًا إلى مجموعة سيطرة صحية (العدد = 40)، مرضى الشريان المحيطي الأساسيين غير المعالجين بـTicagrelor (B–PAD, n=30)، ونفس مرضى الشريان المحيطي بعد علاجهم بـTicagrelor (A–

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PAD, n=30). شملت المعلمات المقاسة تركيز/ فعالية السوبر اوكسيد دسميوتيز، مستويات البروتين المؤكسد، العناصر النزرة في المصل، وغيرها من المعلمات البيوكيميائية. أظهر مرضى الشريان المحيطي ارتفاع مستوى البروتين المؤكسد وانخفاض تركيز وفعالية السوبر اوكسيد دسميوتيز مقابل مجموعة السيطرة. اظهر ال Ticagrelor القدرة على تحسين التوازن التأكسدي في مرضى الشريان المحيطي عبر التنظيم بزيادة السوبر اوكسيد دسميوتيز وتقليل أكسدة البروتين. أظهرت متغيرات الإجهاد التأكسدي/ مضادات الأكسدة إمكانية فحص مرضى الشريان المحيطي وتشخيصه والمراقبة الطولية لاستجابة العلاج باستخدام المقاييس التشخيصية المستمدة من تحليل الخصائص العملياتية للمستقبِل.

1. Introduction

Peripheral Artery Disease (PAD) is a condition characterized by the narrowing of blood vessels due to atherosclerosis. It is estimated to impact nearly 7 to 12 million of adults in the United States and 200 million patients worldwide [1]. PAD is linked to adverse health outcomes, diminished functionality, and reduced quality of life, partly due to the potential need for amputation. Over a 5- to 15-year period following PAD diagnosis, morbidity and mortality rates fall between 30% and 70% [2]. Similar to other conditions in the cardiometabolic category, social determinants of health play a significant role in various aspects of PAD, including its prevalence, optimization of perioperative medical care, utilization of diagnostic testing, and choice of surgical approach [3, 4]. Asymptomatic peripheral artery disease (APAD) in combination with traditional risk factors like hypertension, diabetes mellitus, and smoking significantly increases the risk of cardiovascular complications. Patients suspected of having underlying PAD should undergo further non-invasive tests for proper evaluation [5, 6].

Ticagrelor is a reversible, direct-acting P2Y12 receptor inhibitor with a unique chemical structure that differentiates it from clopidogrel, prasugrel, and inhibitors. P2Y12, one of the two types of purinergic receptors located on the platelets that are activated by adenosine diphosphate (ADP) and thereby involved in ADP-induced platelet aggregation. The P2Y12 signaling amplifies platelet responses to agonists that cause ADP release, stabilizes platelet aggregates, and counteracts the anti-platelet effects of prostacyclin, which increases platelet levels of cyclic adenosine monophosphate (cAMP) via adenylyl cyclase activation [7]. The importance of P2Y12 in hemostasis and thrombosis is evidenced by bleeding in patients with inherited P2Y12 defects and reduced adverse cardiovascular events in patients receiving receptor antagonists [8]. The frequently employed P2Y12 inhibitor is the thienopyridine prodrug Ticagrelor, which exerts irreversible inhibition of P2Y12 once it undergoes hepatic conversion into its active form. In contrast to clopidogrel, Ticagrelor delivers more potent and consistently sustained P2Y12 inhibition. A comparable heightened inhibitory impact is witnessed with the third-generation thienopyridine drug prasugrel, although Ticagrelor demonstrates a modestly superior platelet inhibitory effect in comparison to prasugrel [9].
Ticagrelor remedy substantially accelerated the production of nitric oxide (NO) and the expression of endothelial nitric oxide synthase (eNOS) in oxidized low-density lipoprotein (ox-LDL)-treated human umbilical vein endothelial cells (HUVECs), thereby restoring the antioxidant reputation that become impaired via ox-LDL. Ticagrelor has been shown to shield endothelial cells from oxidative strain because of ox-LDL. This is harmful because oxidative pressure can result in endothelial disorder, which substantially will increase the risks of atherosclerosis and other cardiovascular issues. This mechanism shows Ticagrelor might also offer benefits above and beyond its antiplatelet effects. By improving antioxidant ranges and shielding endothelial cells from oxidative strain, Ticagrelor should assist in lowering the risks of further cardiovascular occasions [10]. As a result of decreasing

antioxidants, reactive oxygen species (ROS) are increased the state known 'oxidative stress' which is mainly contributed in progress of several diseases [11, 12].

Superoxide dismutase (SOD) is an essential antioxidant enzyme found in all human cells. It plays an essential position in coping with various stressors. SODs are metal-containing proteins that are available in different sorts, the maximum common being manganese SOD (Mn-SOD), copper-zinc SOD (Cu/Zn-SOD), iron SOD (Fe-SOD), and nickel SOD (Ni-SOD) [13]. When ROS build up excessively, SOD activity rises to correctly neutralize dangerous oxygen in the cells. That SOD manufacturing will increase in reaction to higher oxidative pressure underscores its key function in bolstering the herbal antioxidant defences and preserving cell stability [14-16].

The aim of this study was to investigate how Ticagrelor treatment may affect SOD levels in patients with PAD. We assessed SOD levels in PAD individuals treated with Ticagrelor versus controls to determine whether antioxidant enzyme generation was affected by Ticagrelor. To assess the potential of SOD as a marker to regulate oxidative status over time, we also studied the relationship between SOD levels and PAD severity. Identification of the combination may establish SOD as an important clinical parameter in PAD progression and response to treatment. Such findings may provide new insights supporting the role of Ticagrelor in oxidative defence against PAD pathology.

2. Materials and methods

2.1. Study population

A total of 70 people participated in the study, divided into three groups. The first group, called the B-PAD group, consisted of 30 subjects (15 men and 15 women) previously diagnosed with PAD but not yet treated with Ticagrelor. The mean age was 55.87 ±8.55 years. These participants were given Ticagrelor 75 mg daily for 5 days, forming the A-PAD group.

The diagnosis of PAD was established through a comprehensive screening process, including medical record review, laboratory testing, and clinical examination by a consultant cardiologist. Blood samples were collected from patients and healthy individuals using 5 ml syringe, dispensed into gel tubes, allowed to clot for 30 minutes at room temperature and were centrifuged at 3000 rpm for 10 minutes. The samples were collected between June to December 2023. All of contributors were informed about the criteria of the research, and their agreement was reported. The Ethics Committee of the Faculty of Sciences of Baghdad University approved the study protocol.

In addition, 40 volunteers (20 males and 20 females) with a mean age of 55.43 ±8.40 years with no history of PAD were included in the study as control group. Demographic and clinical data were collected for both patients and control groups. Exclusions included individuals who drank alcohol, smoked, and were on Ticagrelor therapy prior to admission, or who were taking medications with pre-existing chronic diseases or PAD.

2.2. Evaluation of routine parameters

The concentrations of random blood sugar (RBS), urea, creatine, alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP) in serum were measured using a Cobas c311 auto-analyzer (Roche Diagnostics, Germany). Standard assays were performed according to the manufacturer's instructions to quantify each analyte. The concentrations of total cholesterol (TC), triglyceride (TG) and HDL-C were determined by

using kits from (Abbott, Chicago, Ill) with an Abbott Aeroset auto-analyzer (Chicago, IL, USA), whereas the LDL-C concentration was calculated by the Friedewald formula [17].

2.3. Evaluation of zinc, copper and manganese elements

Quantification of Zinc (Zn) and Copper (Cu) levels in the serum were assessed through spectrophotometric methods employing kits from CENTRONIC GmbH in (Germany). Meanwhile, manganese (Mn) levels in the serum were measured using atomic absorption spectrometry following a 1:1 dilution with deionized distilled water [18].

2.4. Evaluation of carbonyls in oxidized proteins

The proposed method for quantifying carbonyl groups, termed the DNPH alkaline method, involved adding 400 µL of 10 mM of 2,4-Dinitrophenylhydrazine (DNPH) solution in 0.5 M H_3PO_4 to 400 µL of the serum sample. After allowing a 10-minute incubation period, 200 µL of 6 M NaOH solution was introduced. Absorbance was then measured at 450 nm after further 10-minute incubation at room temperature using APEL PD-303 analyzer (Japan) spectrophotometer. The blank consisted of the same reaction mixture without the serum sample which was substituted by an equal volume of buffer solution [19].

2.5. Evaluation of Super Oxide Dismutase Activities

The activities of total SOD (T-SOD), Cu/Zn-SOD, and Mn-SOD in serum were measured using a biochemical assay kit from Elabscience (USA) based on the hydroxylamine method [20]. An enzyme-working solution and chromogenic agent were used to determine T-SOD, while samples were pretreated with extracting solution for measuring of Cu/Zn-SOD by Mn-SOD inhibition. The absorbance was then measured at 550 nm after adding a chromogenic agent and a 10-minute incubation period. According to this method the one unit of enzyme activity is defined as the amount of SOD causing 50% inhibition ratio in 1 mL reaction solution.

2.6. Evaluation of Super Oxide Dismutase concentration

To detect T-SOD concentration, commercially available sandwich-type ELISA Kit (Elabscience, USA) was used following the manufacturer's instructions. The specific activity for each SOD type was calculated by dividing its activity by the SOD concentration.

2.7. Statistical Analysis

The SPSS program, version 22.0, was employed for the statistical analysis of the data. The analytically assessed data was reported as mean with standard deviation (SD). To compare means across all groups, a one-way analysis of variance (ANOVA) and T-test were conducted, followed by the post-hoc least significant differences (LSD) test.

3. Results

Table 1 presents a comparison of demographic and clinical biomarkers between two groups: a group of 40 healthy individuals and a group of 30 patients diagnosed with PAD. No statistically significant differences were found among studied groups in terms of age, body mass index (BMI), ALP, TG and HDL-C. Patients in the PAD group exhibited significantly higher levels of urea and creatine compared to healthy controls, along with higher levels of RBS, TC and LDL-C. However, they exhibited significantly lower levels of ALT and AST compared to healthy individuals. Treatment with Ticagrelor had no significant effect on these parameters in PAD patients, except TC and LDL-C which showed a noticeable decrease to reach their levels in control group, Table 1.

Table 1: Demographic characteristic and biochemical parameters of patients with PAD and healthy individuals.

Parameter	Control group	B-PAD group	A-PAD group	*P*-value
n	**40**	**30**		
Age (year)	55.43±8.4	55.87±8.55		0.830
BMI (kg/m^2)	30.47±4.17	31.06±4.83		0.585
RBS (mg/dL)	97.88±11.58	108.83±22.78	105.73±20.82	0.011[a,b], 0.087[c]
ALT (U/L)	28.78±6.69	11.99±1.68	12.05±1.66	<0.001[a, b], 0.065[c]
AST (U/L)	33.85±6.64	15.75±1.94	15.39±1.71	<0.001[a, b], 0.252[c]
ALP (K.A.U)	76±23.54	84.39±12.54	84.50±11.20	0.081[a, b], 0.823[c]
Urea (mg/dL)	27.25±8.19	35.32±7.07	35.53±7.49	<0.001[a, b], 0.812[c]
Creatine(mg/dL)	0.48±0.12	0.91±0.28	0.87±0.18	<0.001[a, b], 0.078[c]
TC (mg/dL)	184.25±30.42	216.07±54.9	185.87±25.79	0.003[a], 0.233[b], 0.008[c]
TG (mg/dL)	142.6±21.65	148.37±24.25	146.03±21.91	0.299[a], 0.432[b], 0.812[c]
HDL-C (mg/dL)	45.85±3.54	45.63±12.54	46.67±4.39	0.917[a], 857[b], 0. 844[c]
LDL-C (mg/dL)	109.88±29.39	140.76±57.12	112.57±28.44	0.004[a], 0.087[b], 0.005[c]

The results are presented as mean ± SD, *P*-value ≤0.05 is considered to be significant. *P*-value≤0.05 is considered as significant between a (control and B-PAD groups), b (control and A-PAD groups), and c (B-PAD and A-PAD groups).

The results from Figure 1 show a significant decrease in the SOD concentration as well as the activity and specific activity of T-SOD and Mn-SOD in patients with PAD compared to healthy individuals (Figures 1A-C). However, PAD patients exhibited significantly higher Cu/Zn-SOD activity and specific activity as well as oxidized protein concentration (Figures 1B-D). It was observed that treatment with Ticagrelor led to significant increases in SOD concentration, T-SOD and Cu/Zn-SOD activities alongside significant decrease in Cu/Zn-SOD specific activity and oxidized protein concentration compared to non-treated PAD patients.

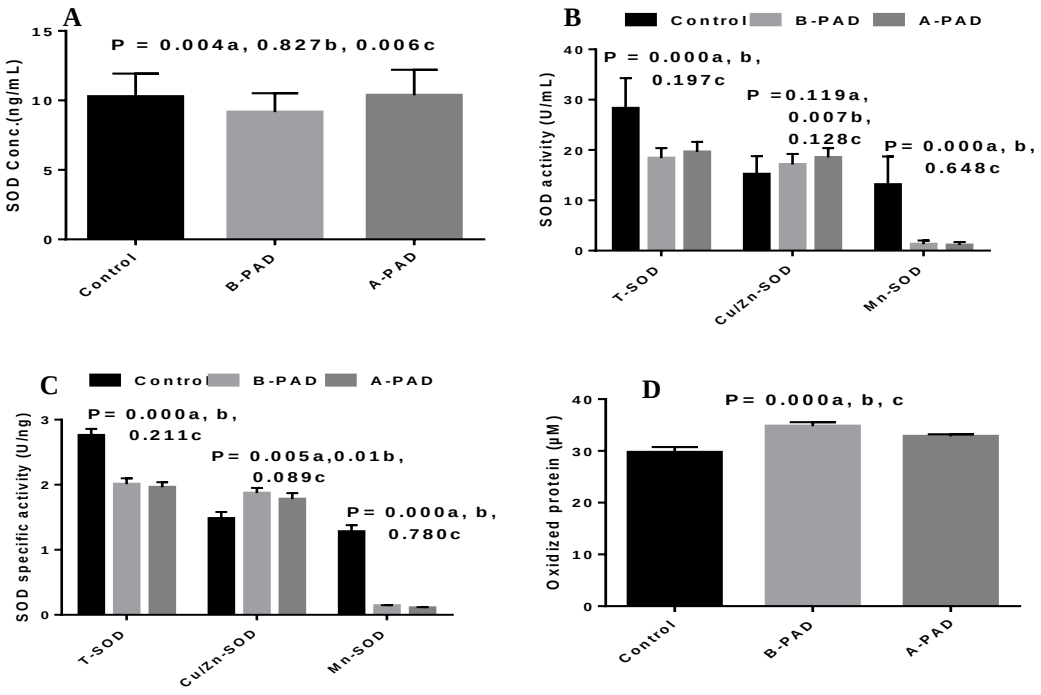

Figure 1: The levels of (A) SODs concentration, (B) SODs activities, (C) SODs specific activities and (D) oxidized protein concentration in healthy individuals and in PAD patients before (B-PAD) and after (A-PAD) receiving of Ticagrelor. P-value< 0.05 is considered as significant between a (control and B-PAD groups), b (control and A-PAD groups), and c (B-PAD and A-PAD groups).

A comparison among the studied groups shows that there is no statistically significant difference in concentration of the trace elements Cu, Zn, and Mn, as shown in Table 2.

Table 2 The concentrations of Cu, Zn and Mn trace elements in control and PAD patients.

Parameter	Control group	B-PAD group	A-PAD group	*P*-value
Cu (mg/dL)	92.7±17.189	96.567±16.823	96.233±17.411	NS
Zn (µg/dL)	85.4±22.204	86.133±23.118	90.9±22.898	NS
Mn (ng/dL)	10.4±3.983	10.323±3.949	10.357±3.925	NS

The results are presented as mean±SD, *P*-value≤0.05 is considered to be significant (NS= non-significant).

Figure 2: presents the results of Pearson correlation analyses between various markers in healthy individuals and PAD patients treated with Ticagrelor. In both control and A-PAD groups, significant positive correlations were observed between SOD concentration and Mn-SOD activity, SOD concentration and Cu concentration, as well as between T-SOD and Cu/Zn-SOD activities. Additionally, SOD concentration exhibited a significant negative correlation with Cu/Zn-SOD in control group, but it is positively correlated with Cu/Zn-SOD in A-PAD. In terms of other parameters, no significant differences in there correlations were observed.

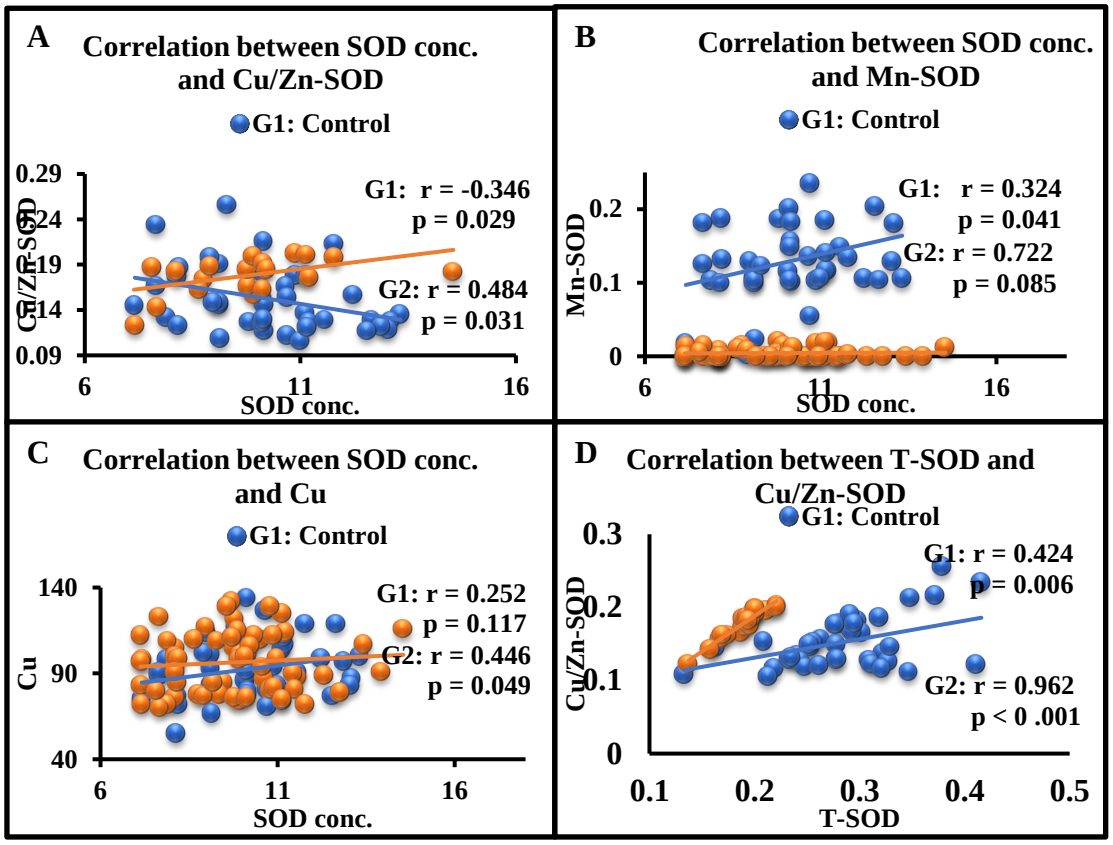

Figure 2: Pearson correlations between (A) SOD concentration and Cu/Zn-SOD activity, (B) SOD concentration and Mn-SOD activity, (C) SOD concentration and Cu, (D) T-SOD and Cu/Zn-SOD activities in control and A-PAD groups.

Figure 3 displays the receiver operating characteristic (ROC) curves for T-SOD activity, Mn-SOD activity, oxidized protein concentration, and Cu/Zn-SOD activity between PAD patients before and after treated with Ticagrelor. The area under the curve (AUC) was 0.930 for T-SOD activity, 0.983 for Mn-SOD activity, 0.993 for oxidized protein concentration, and 0.737 for Cu/Zn-SOD activity, indicating strong discriminatory ability between B-PAD and A-PAD groups.

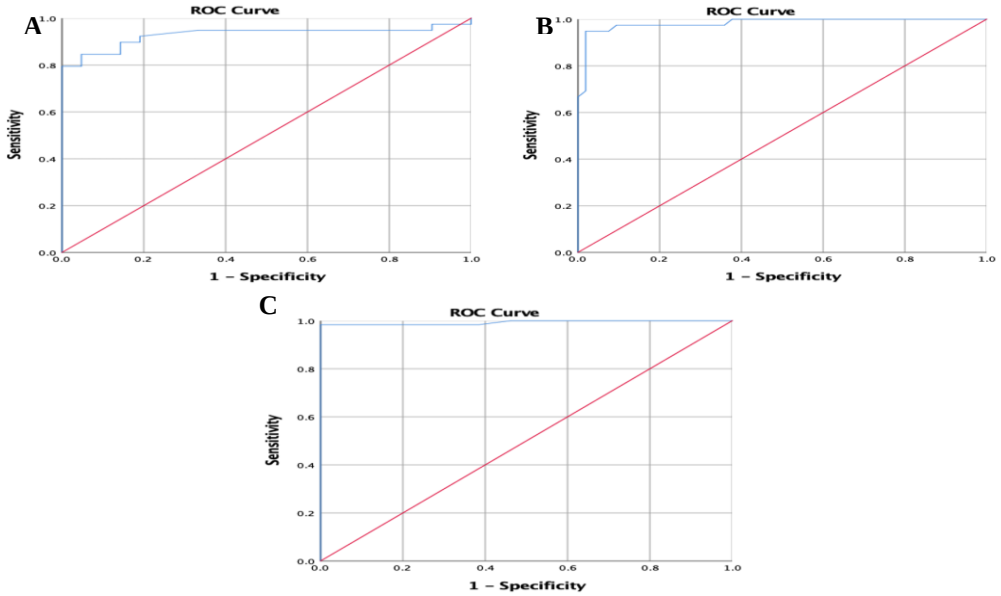

Figure 3: The ROC curves of (A) T-SOD activity, (B) Mn-SOD activity and (C) oxidized protein for discrimination between B-PAD and A-PAD groups.

The optimal cut-off values were determined to be 20.5 U/mL for T-SOD activity, 0.0105 U/mL for Mn-SOD activity and 30.23 μM for oxidized protein concentration, providing diagnostic thresholds for these biomarkers in patients with PAD. The results from the ROC curve analyses for biomarkers that demonstrated meaningful associations with PAD diagnosis in individuals taking Ticagrelor medication are presented in Table 3.

Table 3: ROC results of parameters that showed significant correlations in the diagnosis of PAD patients who received Ticagrelor.

Parameter	AUC	*p*-value	SE	Cut-off value	Sensitivity	Specificity
T-SOD activity	0.930	<0.001*	0.036	20.5	92.3%	81.0%
Mn-SOD	0.983	<0.001*	0.012	0.0105	97.4%	77.4%
Oxidized protein	0.993	<0.001*	0.007	30.23	98.4%	61.5%

Remarkably, the AUC for T-SOD activity, Mn-SOD activity and oxidized protein concentration were all highly statistically significant at $p<0.001$, demonstrating strong potential as diagnostic indicators. According to our results, these parameters can accurately discriminate between PAD and PAD patients receiving Ticagrelor, with Mn-SOD activity and oxidized protein concentration proving to be the most diagnostically accurate based on their test performance.

4. Discussion

Table 1 shows there were no significant differences in age, BMI, ALP, TG and HDL-C parameters between PAD patients and healthy controls. PAD patients had higher levels of RBS, urea, creatine, TC and LDL-C and low levels of ALT and AST compared to healthy individuals. This suggests in PAD patients the kidney and liver function may be affected by this pathogenesis condition. After receiving Ticagrelor these parameters did not show significant change, however, TC and LDL-C levels were noticeably improved. These findings align with prior researches [21-23]. Sarmento *et al*. found PAD patients had significantly elevated ox-LDL, malondialdehyde levels and lower SOD, glutathione peroxidase levels versus controls. They also found ox-LDL and malondialdehyde positively correlated with urea and creatine, implying oxidative stress associates with renal dysfunction in PAD [24].

The significant differences in SODs concentration, SODs activities, SODs specific activities, and increased oxidized protein concentration between healthy individuals and PAD patients suggest that oxidative stress is elevated in PAD patients (Figure 1). The SODs are antioxidant enzymes that help protect against free radical damage, however lower SODs levels and activities in PAD fail to adequately neutralize oxidative species, worsening stress. Oxidized proteins result from radical attack on biomolecules, with higher amounts in PAD linked to cellular harm and death. A meta-analysis of 10 studies reported PAD patients had significantly higher reactive oxygen species (ROS) and lower SOD, glutathione peroxidase, catalase antioxidants than controls. The ROS levels also positively correlated with urea and creatine, proposing oxidative stress contributes to kidney impairment in PAD [25]. Collectively, this research indicates PAD patients experience impaired renal function, heightened oxidative stress and depleted antioxidant defences, which could link to disease development and progression. The current study aligns with prior work demonstrating PAD-associated oxidative imbalance and kidney implications [26-28]. Prior work shows diminished antioxidants like SOD and glutathione peroxidase coupled with heightened ROS and malondialdehyde in PAD versus controls [29, 30]. Oxidative stress drives PAD progression via DNA/protein damage, inflammation, coagulation and atherosclerosis

acceleration [31]. While Ticagrelor therapy boosted SODs and decreased oxidized proteins in PAD, signifying protective effects, oxidative imbalance commonly occurs in PAD. Also, Ticagrelor's antioxidant activities may slow disease development and reduce complications like heart attack and stroke. Based on *in vitro* study, Meini K. and his colleagues (2019) reported that Ticagrelor has antioxidant activity [10]. Additionally, parameters like low SODs and high oxidized protein levels could aid PAD diagnosis and monitoring, corroborating the clinical importance of oxidative stress in this condition.

The results in Table 2 examine concentrations of the trace elements Cu, Zn and Mn in the healthy individuals, B-PAD as well as A- PAD groups. When analyzing Cu, Zn, Mn levels, there were no significant differences in the levels of these elements among the studied groups. This suggests the results provide no evidence of a relationship between trace element levels and SOD activity. Specifically, statistical analysis failed to demonstrate any correlation between the measured trace element proportions and observed SOD antioxidant activity. The consistent trace element profiles indicate they were not altered by variable mineral quantities within the typical physiological range. These findings did not support trace elements as determinants of SOD activity under the conditions examined. These results agree with previous study conducting on patients with breast cancer [15].

Figure 2 presents Pearson correlations between various markers in control and A-PAD patients. In both control and A-PAD groups, positive correlations were seen between SOD concentration and Mn-SOD activity and Cu levels, suggesting these indicators increase together. Total SOD activity also positively correlated with Cu/Zn-SOD activity. The results of Figure 2 are consistent with other related studies. Wang *et al*. (2020) similarly found positive correlations between SOD concentration and Cu/Zn-SOD activity, Mn-SOD activity, and copper levels in patients with coronary artery disease [32]. The interrelationships identified between SOD, enzyme activities, Cu levels, and protein markers in this study parallel the findings of Zhang *et al*. and Pandey *et al*., suggesting shared oxidative stress pathways may underlie these relationships [32, 33]. Overall, the correlations observed provide further support for the role of oxidative imbalance in PAD pathogenesis, aligning with previous evidence presented in references.

Table 3 used ROC curve analysis to evaluate the diagnostic performance of three major parameters: T-SOD activity, Mn-SOD activity, oxidized protein levels, and Cu/Zn-SOD activity. ROC analysis revealed a significant association for each parameter in PAD patients treated with Ticagrelor compared to untreated PAD patients. Receiver operating characteristic curve analysis provides an effective method for evaluating the performance and clinical utility of biomarkers for the detection and management of PAD, as shown in Table 3. The area under the ROC curve (AUC) indicates how well a test can discriminate between patients before and after treatment with Ticagrelor, higher values are closer to showing better discrimination. The results displayed in Table 3 indicate that all of the listed parameters exhibited high areas under the curve (AUCs), implying that these parameters have significant potential for guiding and optimizing Ticagrelor treatment regimens. Importantly, the sensitivity and specificity values derived from ROC analysis at optimal cut-off thresholds established the clinical effectiveness of these markers. Tests with high sensitivity, such as Mn-SOD and oxidized protein, are well-suited for initial PAD diagnosis since they can detect true positive cases while minimizing missed diagnoses. Meanwhile, markers with a balance of high sensitivity and specificity, such as T-SOD activity, help to rule out false positives and confirm the presence of disease monitoring changes in biomarker levels so sequentially in response to treatments supports clinical research, ROC analysis provides a framework for identifying the most objectively valid and clinically useful markers for both PAD diagnosis and long-term treatment response research provides, thereby enhancing patient care and management outcomes.

Conclusion

In conclusion, this study showed that patients with PAD exhibit increased oxidative stress and decreased antioxidant defence evidenced by lower T-SOD and Mn-SOD levels and increased protein oxidation compared with controls. Treatment with Ticagrelor improves oxidative imbalance by increasing SOD levels, biomarkers of oxidative stress represented by oxidized protein and SOD antioxidants were found to be associated with PAD high correlation and showed high diagnostic accuracy for disease detection based on ROC analysis. The results provide additional insights supporting the ability of Ticagrelor to clinical utility to improve PAD diagnosis, screening, and treatment response. Also, monitoring long-term -Ticagrelor modulation of the system may help slow the progression of PAD and help reduce associated complications. Overall, this study provides valuable information on the effects of Ticagrelor on the antioxidant system and its implications for the optimal management of individualized PAD using antioxidant biomarkers.

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