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The Gamma-Glutamyl Transferase-Malondialdehyde-Nitric Oxide Nexus: A Proposed Vascular Uncoupling Index for Endothelial Dysfunction in Hypertension

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ABSTRACT

Redox imbalance influences biochemical changes linked with the pathogenesis of hypertension. This study assessed the levels of systolic blood pressure (SBP), diastolic blood pressure (DBP), body mass index (BMI), gamma-glutamyl transferase (GGT), Malondialdehyde (MDA), Nitric Oxide (NO) and computed mathematical indices to determine whether a model integrating the biomarkers at a certain threshold can serve as a biochemical fingerprint for endothelial damage in hypertensives. Fifty hypertensive subjects attending Clinics at University of Calabar Teaching Hospital, Calabar, and 40 healthy age-matched controls were recruited into this cross-sectional study. The GGT, MDA and NO were determined by respective colorimetric methods, height and weights were measured and BMI computed. Blood pressure was measured using a sphygmomanometer. Data generated were analyzed using SPSS version 27, (IBM). Differences between groups were determined by Student's T-test. The Receiver Operator Characteristic Curve was utilized to determine the thresholds of biochemical indexes predictive of redox- imbalance and endothelial dysfunction. The confidence interval was set to 95% at α =0.05. The SBP, DBP, GGT, MDA, MDA/NO, GGT/NO and VUI levels were significantly higher, while NO was significantly lower in hypertensives when compared with normotensives ($p<0.05$). At a threshold of 0.43, vascular uncoupling index (VUI) demonstrated AUC, Gini index, maximum K-S metric of 0.993, 0.986, 0.920, sensitivity and specificity of 92% and 100% (95%C.I 0.983-1.003) respectively. The MDA correlated negatively with NO (r= -0.767, p=0.000, 95%C.I -0.859-0.616). Limitations include lack of subgroup analysis by antihypertensive drug class and a single-center design. The findings of this study suggest that Vascular Uncoupling Index/Redox Imbalance Score, may be more strongly associated with endothelial dysfunction in hypertension than GGT, MDA, and NO assessed discretely. Targeted adjunctive interventions decoupling the GGT-MDA-NO nexus in hypertensives may rescue endothelial dysfunction, proposing a non-canonical pathway for blood pressure reduction but requires validation in an independent cohort.

Keywords: Redox imbalance, hypertension, endothelial dysfunction

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1.0 Introduction

Hypertension (high or raised blood pressure) is a condition where the blood vessels insistently experience raised pressure, systolic pressure of 140 mmHg or higher and a diastolic pressure of 90 mmHg or higher. The gamma glutamyl transferase (GGT)-malondialdehyde (MDA)-nitric oxide (NO) nexus may represent a critical redox triangle driving endothelial dysfunction (ED), an essential, beginning event in the pathogenesis and progression of hypertension and cardiovascular disease (Gallo *et al.,* (2022; Ortakoyluoglu *et al.,* 2016). It illustrates the progression from metabolic stress to systemic vascular dysfunction. The NO is the primary vasodilator responsible for preserving vascular tone. The GGT-MDA-NO axis operates in a feedforward loop. Elevated serum GGT levels signify early signs of oxidative stress and a pro-oxidant state that promotes lipid peroxidation (increased MDA production) and disrupts endothelial function with reduced nitric oxide (NO), eventually damaging vascular health, perpetuating the cycle (Eruyar *et al.,* 2025; Antwi-Boasiako, *et al.,* (2020). An estimated 1.4 billion adults within the ages of 30–79 years globally had hypertension in 2024; representing 33% of the population in this age range (WHO 2025). Hypertension is responsible for 8·5 million deaths from stroke, ischaemic heart disease, other vascular diseases worldwide (Zhou *et al*, 2021). Whether targeted adjunctive interventions decoupling the GGT-MDA-NO pro-oxidant pathway can rescue endothelial dysfunction in hypertensives is not fully understood. Also, whether a mathematical model integrating these three markers at a certain threshold can serve as a biochemical fingerprint for endothelial damage is not clear. Current literature often treats these markers as linear predictors. This study proposes a non-linear tripartite model to identify the precise threshold where GGT- MDA-NO becomes a pro-oxidant driver of endothelial dysfunction. This study tested two hypotheses (1) a composite "Redox Imbalance Score" (RIS), or Vascular Uncoupling Index (VUI), calculated from GGT-MDA-NO is more strongly associated with endothelial damage and resistant hypertension than any of the three markers treated separately, (2) a high GGT activity, in the incidence of low NO bioavailability, serves as a catalyst for the copper or iron-catalyzed Haber-Weiss reaction within the endothelium. The GGT-MDA-NO "Oxidative Triangle" signifies a vicious, highly unified pathophysiological loop that drives endothelial dysfunction and vascular remodeling in arterial hypertension. The GGT-MDA-NO activities form a self-sustaining tri-directional axis where changes in the activities of one component of the triangle inescapably triggers the increase or diminution in the levels of the other components of the vertices of the triangle. Each of the three vertices (A, B, C) of the triangle may be considered as being occupied arbitrarily by one of the biomarkers GGT, MDA, or NO respectively

At Vertex A of the oxidative triangle is GGT; the pro-oxidant catalyst, that maintains cellular glutathione (GSH) reserves, elevated circulating or membrane-bound GGT acts as a potent pro-oxidant within the vascular wall. The GGT cleaves extracellular GSH into cysteinyl-glycine (Ford and Miranda, 2020; Balazinski *et al.,* 2021). Find detailed chemical mechanisms in supplementary materials.

At Vertex B of the oxidative triangle, Malondialdehyde (MDA), the propagation vector, is a reactive dialdehyde generated as a stable end-product of lipid peroxidation. Lipid peroxidation is a free-radical chain reaction that degrades polyunsaturated fatty acids (PUFAs) in cell membranes to form malondialdehyde (MDA) (Zheng *et al.,* 2024). The MDA serves both as a measurable footprint of this oxidative damage, as well as an active propagator of vascular injury (Asfandiyar *et al.,* 2024; Černiauskas *et al.,* 2023). Find detailed chemical mechanisms in supplementary materials.

At Vertex C is NO, the Depleted Vasodilator, synthesized by endothelial nitric oxide synthase (eNOS), is the fundamental mediator of vasodilation, anti-platelet aggregation, and vascular smooth muscle relaxation (Łuczak *et al.,* 2020). The NO is rapidly scavenged in a high ROS (high MDA and GGT) environment. This study assessed the levels of GGT, MDA, NO and computed mathematical indices to determine whether a model integrating these three markers at a certain threshold can serve as a biochemical

fingerprint for endothelial damage in hypertensives.

2.0 Materials and Method

Study design

This study was a cross-sectional design conducted in Calabar Metropolis from October 2023 to August 2024, among subjects with hypertension attending Clinics at the University of Calabar Teaching Hospital, Calabar. The hypertensive subjects were further categorized into hypertensives with normal BMI and overweight hypertensives

Study setting/Subject selection

A total of 90 comprising of 50 hypertensives aged 36-65 years and forty apparently healthy age-matched and individuals with no significant difference in BMI, residing in the same geographic location, screened for diabetes and liver diseases, confirmed to have normal blood pressure (normotensives), who served as controls were recruited into the study.

Inclusion and Exclusion criteria

Hypertensive subjects between the ages of 36-65 years, not critically ill, who gave consent, those with no liver diseases were recruited into this study. While subjects with history of smoking and alcohol use, comorbidity with Type 2 Diabetes were excluded from the study

Sample collection

Five milliliters of venous blood was collected aseptically from each subject by venepuncture after an overnight fast, dispensed into plain bottles allowed to clot, dislodged and spun in the centrifuge at 3000rpm for 5 minutes. The serum was transferred into a 5mls plain serum bottle, correctly labeled and stored frozen at -4^0C until the time for analysis.

Data Collection

Sociodemographic data were collected from the participants using a well-structured interviewer questionnaire.

Sample size calculation

Sample size was determined according to the method of Sullivan (2020)

$$n = \frac{(Z_\alpha + Z_\beta)^2 \cdot \bar{p}(1-\bar{p})}{(p_0 - p_1)2}$$

- Z_β= Beta error at 80% power is 0.84
- Z_α = Z value associated with 95% confidence level is 1.96, ie, (Alpha error at 95% level of confidence)
- P_0= The anticipated probability of "exposure for people with the disease condition" (97%=0.97)
- P_1= is the proportion/anticipated probability of "exposure for people without the disease condition" 86% = 0.86 (estimated from the postulated odds ratio)
- ṗ=mean proportion between cases and control group $(p_1+p_2)/2$ =0.97+0.86)/2=0.91

d = difference to be detected

$(p_0 – P1) = 0.97-0.86=0.11$

$$n= \frac{(1.96+0.84)^2 X\ 0.91(0.09)}{(0.11)^2} = 50$$

A minimum sample of 52 hypertensives is required, our sample of 50 hypertensives subjects is a limitation

Anthropometric indices

The weight, in kilograms (kg) and height in meters (m) of the subjects were measured using the bathroom scale and Stadiometer respectively. Body mass index (BMI) was computed as the ratio of weight in kilogram (kg) to height in meters squared (m^2). Systolic and diastolic blood pressure were measured in millimeter mercury (mmHg), using the sphygmomanometer.

Statistical analyses

Data generated were analyzed using SPSS version 25 (IBM Inc.). Results are presented as Mean± standard deviation. The normality of the data was determined by Shapiro-Wilk test and Box and Wisker plots. Student's t-test and Pearson's correlation was utilized in determining mean difference between groups associations between variables respectively.

The Receiver Characteristic Operator Curve (ROC) analysis was utilized to determine the

performance metrics of the biomarkers and biochemical indices in predicting outcomes in the test subjects. A logistic regression model incorporating GGT, MDA, and NO was compared with VUI alone using DeLong's test for correlated AUCs. Internal validation was performed using 10-fold cross-validation to estimate corrected AUC. Given multiple comparison, statistical significance was evaluated using Bonferroni-corrected threshold ($p<0.0025$). The level of significance was set at $p<0.05$.

Laboratory methods

Gamma glutamyl transferase was estimated using colorimetric enzymatic assay according to the SZAZS method 1976, using a kit obtained from Fortress Diagnostics Limited, Antrim, BT41 1QS (United Kingdom). Nitric oxide was determined by Griess colorimetric method, using reagent kit by Promega Fitchburg, USA. Malondialdehyde (MDA) was determined by Thio-Barbituric Acid Reactive Substances (TBARS) method, using a kit from ABCAM Cambridge, UK. The TBARS method overestimates MDA due to interference from other aldehydes; HPLC or LC-MS/MS is preferred.

The MDA: NO, GGT: MDA and GGT:NO were computed as ratios between the two markers.

Vascular Uncoupling Index (VUI) was computed using the formula

$$VUI = \frac{GGT \times MDA}{NO}$$

Ethical Consideration

Ethical approval was obtained from Ethics and Research Committee of the Cross River State Ministry of Health with the approval number: *CRSMOH/HRP/REC/2023/470*. Informed consent was obtained from all participants. The research was conducted according to the Helsinki's declaration about research involving Human subjects (1975), and as revised subsequently.

3.0 Results

A comparison of age, blood pressure, BMI, GGT, MDA, NO, MDA/NO, GGT/MDA, GGT/NO and VUI between hypertensives and normotensives is shown in table 1. The SBP, DBP, GGT, MDA, MDA/NO, GGT/NO and VUI levels were significantly higher, while NO was significantly lower in hypertensives when compared with normotensives ($p<0.05$). The GGT/MDA ratio was not significantly different between the two groups ($p>0.05$).

The comparison of age, blood pressure, BMI, GGT, MDA, NO, MDA/NO, GGT/MDA, GGT/NO and VUI between hypertensives with normal BMI and overweight hypertensives is represented in table 2. The MDA was significantly higher in overweight hypertensives when compared with hypertensives with normal weight ($p<0.05$). The Age, SBP, DBP, GGT, NO, MDA/NO, GGT/MDA, GGT/NO and VUI levels were not significantly different between the two groups ($p>0.05$).

The Receiver Operator Characteristic Curve of MDA, NO, GGT, VUI, MDA/NO ratio, GGT/NO ratio, GGT/MDA ratio in hypertensives is shown in figure 1. At a threshold of 0.43; vascular uncoupling index demonstrated AUC, Gini index, maximum K-S metric of 0.993, 0.986, 0.920, and a sensitivity and specificity of 92% and 100% respectively. The optimal cutoff (0.43) was selected by maximizing Youden's index (sensitivity + specificity – 1), which also aligns with the maximum Kolmogorov-Smirnov (KS) index. GGT shows near-perfect diagnostic capability with an AUC of 0.998. Utilizing a cutoff value of > 25.21, it achieves a flawless Specificity (100%) and 98% Sensitivity. VUI (AUC = 0.999) and NO (AUC = 0.985) are also exceptionally strong classifiers. Note that NO is inversely associated with the disease state (Cases exhibit lower values of NO, hence the cutoff condition <56.97). The MDA/NO serves as an excellent diagnostic metric (AUC= 0.978). At a threshold of 0.44, GGT/NO emerges as an exceptional composite marker (AUC = 0.994). By taking the powerful upward push of GGT in cases and compounding it with the diminution in NO, this ratio maximizes separation, netting 100% Specificity and a 100% PPV. A summary of the performance metrics of the variable studied in hypertensives is represented in table 3.

A correlation plot of GGT against MDA in hypertensives is represented in figure 2. The GGT correlated positively with MDA in hypertensives ($r= 0.678$, $p=0.000$, C.I (0.488-0.802). A

correlation plot of GGT against NO in hypertensives is shown in figure 3. The GGT correlated negatively with NO (r= -0.624, p=0.000, C.I -0.767—0.414). A correlation plot of MDA against NO in hypertensives is depicted in figure 4. The MDA correlated negatively with NO (r= -0.767, p=0.000, C.I -0.859-0.616).

Table 1: Comparison of age, blood pressure, BMI, GGT, MDA, NO, MDA/NO, GGT/MDA, GGT/NO and VUI between hypertensives and normotensives.

Parameters	Hypertensives (HTs) (n=50)	Normotensives (NTs) (n = 40)	T	P	Cohen's d	95%C.I
Age (years)	50.10 ± 8.61	51.00 ± 8.55	0.491	0.625	8.56	4.33-2.88
SBP (mmHg)	155.94 ± 6.54	119.03 ± 5.20	29.078	<0.001*	5.98	34.39-39.44
DBP (mmHg)	102.48 ± 6.46	75.83 ± 5.35	20.955	<0.001*	5.99	24.13-29.18
BMI (kg/m²)	25.60 ± 0.92	25.50 ± 1.04	0.471	0.639	0.98	-0.31—0.51
GGT (U/L)	47.80 ± 13.08	16.07 ± 4.46	14.662	<0.001*	10.20	27.43-36.03
MDA (µmol/L)	1.93 ± 0.65	0.89 ± 0.30	9.371	<0.001*	0.53	0.82-1.27
NO (µmol/L)	46.46 ± 9.18	71.47 ± 10.72	11.923	<0.001*	9.88	-29.17-20.80
MDA/NO	0.05 ± 0.03	0.01 ± 0.00	7.765	<0.001*	0.02	0.25-0.04
GGT/MDA	27.20 ±13.02	21.82 ±14.25	1.865	0.065	13.57	-0.35-11.09
GGT/NO	1.12 ± 0.56	0.24 ± 0.09	9.957	<0.001*	0.42	0.71-1.06
VUI	2.45 ±2.04	0.20 ±0.08	6.968	<0.001*	1.52	1.61-2.89

Key: Results expressed as mean ±SD, SBP=Systolic blood pressure, DBP=Diastolic blood pressure, BMI= Body Mass Index, GGT= gamma-glutamyl transferase, MDA =Malondialdehyde, NO= Nitric oxide, MDA/NO= Malondialdehyde:Nitric oxide ratio, GGT/MDA= gamma-glutamyl transferase:Malondialdehyde ratio, GGT/NO= gamma-glutamyl transferase:Nitric oxide ratio, VUI=Vascular Uncoupling Index

Table 2: Comparison of age, blood pressure, BMI, GGT, MDA, NO, MDA/NO, GGT/MDA, GGT/NO and VUI between hypertensives with normal BMI and overweight hypertensives.

Variable	HTs Normal BMI (n = 16)	Overweight HTs (n = 34)	T	P	Cohen's d	95%C.I
Age (years)	46.43 ± 6.79	51.53 ± 8.90	1.931	0.059	4.68	1.23-0.02
SBP (mmHg)	154.50 ± 7.01	156.50 ± 6.36	0.970	0.337	6.57	-0.92-0.32
DBP (mmHg)	100.79 ± 7.03	103.14 ± 6.21	1.160	0.252	6.52	-0.98-0.25
GGT (U/L)	44.92 ± 14.50	48.92 ± 12.52	0.972	0.336	13.20	-0.93-0.32
MDA (µmol/L)	1.62 ± 0.64	2.05 ± 0.63	2.181	0.034*	0.66	1.32—0.05
NO (µmol/L)	48.77 ± 9.66	45.56 ± 8.96	1.115	0.270	9.21	-0.27-0.97
MDA/NO	0.04 ± 0.03	0.05 ± 0.03	1.364	0.179	0.03	-0.009-0.02
GGT/MDA	29.76 ±8.52	26.21± 14.38	0.863	0.392	13.07	-11.06-4.88
GGT/NO	1.02 ± 0. 59	1.16 ± 0. 54	0.806	0.424	0. 50	-0.22-0.46
VUI	1.94 ± 1.94	2.65 ± 2.06	1.102	0.276	1.81	-0.86-1.50

Key: Results expressed as mean ±SD, SBP=Systolic blood pressure, DBP=Diastolic blood pressure, BMI= Body Mass Index, GGT= gamma-glutamyl transferase, MDA =Malondialdehyde, NO= Nitric oxide, MDA/NO= Malondialdehyde:Nitric oxide ratio, GGT/MDA= gamma-glutamyl transferase:Malondialdehyde ratio, GGT/NO= gamma-glutamyl transferase:Nitric oxide ratio, VUI=Vascular Uncoupling Index, HTs= hypertensives

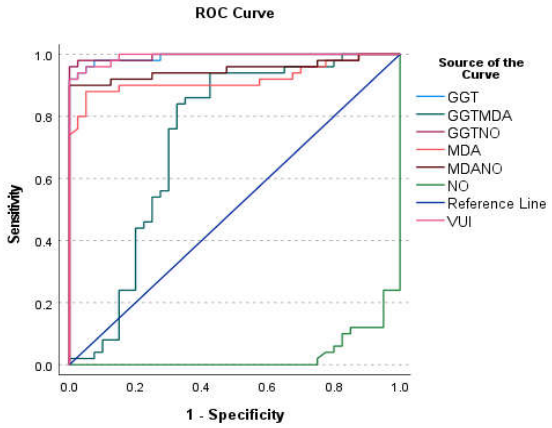

Figure 1: The Receiver Operator Characteristic Curve of MDA, NO, GGT, VUI, MDA/NO ratio, GGT/NO ratio, GGT/MDA ratio in hypertensives.

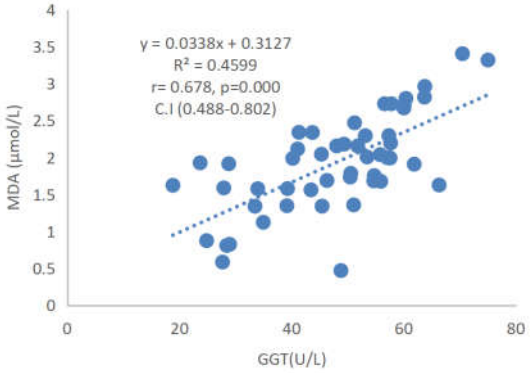

Figure 2: Correlation plot of GGT against MDA in hypertensives

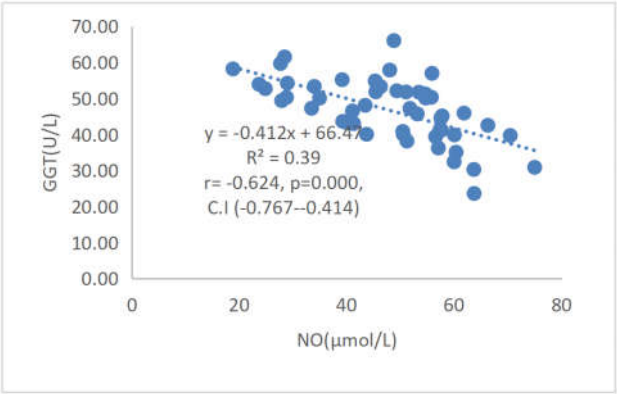

Figure 3: Correlation plot of GGT against NO in hypertensives

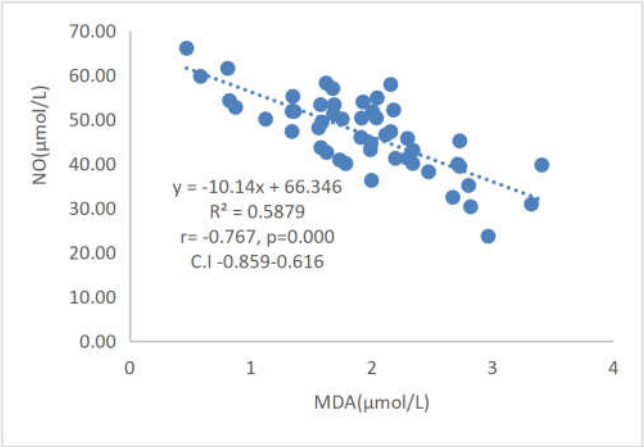

Figure 4: Correlation plot of MDA against NO in hypertensives

Table 3: The performance evaluation metrics of the variable studied in hypertensives

Metric / Biomarker	AUC	S. E	95%C. I	p	Gini Index	Max K-S	Optimal Cutoff	Sen. (%)	Spec (%)	PPV (%)	NPV (%)	Bootstrapped AUC (Mean)	LOOCV AUC
GGT	0.994	0.006	0.982- 1.000	<0.0001	0.988	0.960	> 24.83	96.0	100.0	100.0	96.2	0.993	0.988
MDA	0.887	0.038	0.812-0.962	<0.0001	0.774	0.680	> 1.34	72.0	96.0	94.7	77.4	0.885	0.871
NO	0.985	0.040	0.046-0.202	<0.0001	-0.752	0.780	< 51.26	86.0	92.0	91.5	86.8	0.125	0.119
MDA/NO	0.932	0.027	0.879-0.985	<0.0001	0.864	0.780	> 0.025	86.0	92.0	91.5	86.8	0.930	0.918
GGT/MDA	0.725	0.054	0.619- 0.831	<0.0001	0.450	0.440	> 22.14	74.0	70.0	71.2	72.9	0.722	0.698
GGT/NO	0.994	0.005	0.984-1.000	<0.0001	0.988	0.960	> 0.443	96.0	100.0	100.0	95.2	0.993	0.991
VUI	0.999	0.001	0.997-1.000	<0.0001	0.998	0.980	> 0.445	98.0	100.0	100.0	98.0	0.999	0.996

GGT= gamma-glutamyl transferase, MDA =Malondialdehyde, NO= Nitric oxide, MDA/NO= Malondialdehyde:Nitric oxide ratio, GGT/MDA= gamma-glutamyl transferase:Malondialdehyde ratio, GGT/NO= gamma-glutamyl transferase:Nitric oxide ratio, VUI=Vascular Uncoupling Index

4.0 Discussion

The elevated levels of GGT, MDA, MDA/NO, GGT/NO and VUI and reduced NO in hypertensives when compared with normotensives suggest an underlying oxidative stress partly mediated by GGT. Reactive oxygen species produced during oxidative stress attacks the polyunsaturated fatty acids in cell membranes,

leading to lipid peroxidation, with elevated MDA. Superoxide anions react with NO to form peroxynitrite ($ONOO^-$), a pro-oxidant, which reduces the bioavailability of NO. The loss of NO compromises the vessels' ability to dilate, consequently increasing peripheral resistance and elevating blood pressure. The simultaneous elevated levels of GGT and MDA may serve as an "oxidative bridge" triggering progression from prehypertension to clinical hypertension. Also, a high GGT/MDA ratio may correlate with the speed of arterial remodeling/stiffness in hypertensives. The AUC of GGT/MDA dampens the single performance of GGT considerably, proving that MDA does not share a synergistic diagnostic direction when placed in the denominator. These findings are similar to that of Zuin *et al.,* (2022), who reported elevated MDA to indicate free radical damage on cell membranes, contributing to arterial hardening, worsening hypertension. Similarly, Ortakoyluoglu *et al.,* (2016), reported the association of serum GGT levels (marker of inflammation and endothelial dysfunction), with the worsening of the diurnal rhythm of BP in hypertensives. Also, Ataei Ataabadi *et al.,* (2020), da Silva *et al.,* (2021); observed that increased oxidative stress (indicated by high MDA and GGT) leads to the oxidation of tetrahydrobiopterin (BH4), a cofactor for the enzyme, endothelial nitric oxide synthase (eNOS) required for the synthesis of NO. This results in the uncoupling of eNOS, which then produces superoxide anions that damage the endothelium instead of NO that protects it. The significantly higher levels of the redox imbalance score (RIS) or Vascular Uncoupling Index (VUI) in hypertensives (HTs) compared with normotensives (NTs), suggest considerable redox imbalance in hypertensives, associated with a high GGT and MDA and reduced NO. Operating in a feed-forward loop, GGT mediates the cleavage of glutathione releasing cysteinyl-glycine, which reduces Fe3+ to Fe2+, producing superoxide radicals that further deplete NO required for vasodilation and hastens lipid peroxidation (MDA) as well. This finding is similarly to those of Amponsah-Offeh *et al.,* (2023); Ma *et al.,* (2025); Ranasinghe *et al.,* (2024); Salem and Robenson (2025); Zuin *et al.,* (2022, who reported oxidative stress and redox imbalances in hypertensives. Widespread lipid peroxidation may disrupt the packed structure of the cell membrane lipid bilayer, altering the structural and functional landscape of the cell, affecting membrane fluidity and increasing permeability. The transformed membrane structure impairs membrane-bound pumps, such as Na^+-K^+-ATPase and Ca^{2+}-ATPase, leading to intracellular calcium overload.

The VUI at a cutoff point of 0.445 demonstrated an AUC of 0.999, suggesting that this proposed non-linear tripartite model is highly accurate in identifying the precise threshold where this index predicts the presence of plague, irresistible arterial stiffness, endothelial dysfunction than each of the biochemical markers treated individually. The VUI fingerprint when validated in an independent cohort, may provide a diagnostic score that identifies "biochemical hypertension", allowing for lifestyle modification before vascular remodeling occurs. The VUI representing the net effect of the operations of the GGT-MDA-NO Oxidative Triangle signifies a vicious, highly unified pathophysiological loop that is serves as molecular drivers of endothelial dysfunction and vascular remodeling in arterial hypertension. When the activities of this arbitrary triangle become locked in a positive feedback loop within the vascular bed, it may fuel the progression of hypertensive vascular disease through the loss of vascular compliance. This occurs when a significant reduction in NO bioavailability modifies the vascular tone in favor of chronic vasoconstriction, elevating systemic blood pressure. The activities of the oxidative triangle also result to arterial remodeling vascular smooth muscle cell (VSMC) proliferation and migration, transforming efficient elastic arteries into rigid, remodeled vessels. It also accelerates atherogenesis, when a lack of cytoprotective NO accelerates plaque formation and instability in hypertensive patients. Looking at the GGT-MDA-NO Oxidative Triangle as an integrated system, it becomes clear that targeting just one vector (e.g., trying to supplement NO pathways without vindicating GGT-driven metal reduction or lipid peroxidation) is hardly sufficient to disrupt the pathophysiology of chronic hypertension. These observations are in line with previous studies by Tao *et al.* (2025); Preechakul

et al. (2025), which demonstrated association between hypertension and oxidative balance score.

Comparing the existing endothelial dysfunction markers such as Flow-Mediated Dilation (FMD), Asymmetric Dimethylarginine (ADMA), and Reactive Hyperemia Index (RHI), with a proposed composite vascular uncoupling index (VUI) derived from VUI= (GGT x MDA)/NO), may reveal distinct mechanistic, diagnostic, and practical differences. Flow-Mediated Dilation (FMD), assesses endothelial function by measuring the percentage increase in brachial artery diameter following transient ischemia, inducing vasodilation mediated by endothelial nitric oxide synthase (eNOS) and subsequent NO release. It has the limitations of being highly technician-dependent, requires specialized ultrasound equipment, and is susceptible to acute environmental variations (e.g., temperature, caffeine, etc). Asymmetric Dimethylarginine (ADMA), an endogenous chemical that competes directly with L-arginine to inhibit eNOS, effectively uncoupling the enzyme and shifting its production from NO to superoxide radicals. Elevated ADMA indicates a biochemical blockade of the vasodilatory pathway, strongly correlating with chronic vascular remodeling and severe hypertension. Its limitations include measurement of an upstream inhibitor rather than active vascular output; requires specialized assays (HPLC) that are not routinely available. Reactive Hyperemia Index (RHI), assessed via Peripheral Arterial Tonometry (PAT), RHI measures digital pulse amplitude changes during reactive hyperemia, provides an index of microvascular endothelial function (Sara *et al.,* 2024; Kim *et al.,* 2023). It is influenced significantly by non-NO pathways making it less specific to pure nitric oxide bioavailability. FMD and RHI require expensive equipment and dedicated patient appointments. GGT, MDA, and NO can be analyzed using accessible spectrophotometric and ELISA kits, facilitating easy longitudinal tracking. Also, while a depressed FMD tells you the endothelium is broken, it doesn't tell you why. A high RIS specifically implicates an imbalance between lipid peroxidation and nitric oxide depletion. Potential interventions to test in future studies include GGT inhibitors (e.g., GGsTop), N-acetylcysteine, or BH4 supplementation.

Limitation of the study

Hypertensive subjects on drugs such as statins, known to reduce oxidative stress and MDA were not considered as separate groups. Also subjects taking ACE Inhibitors/Receptor Antagonists (ARBs), that can improve NO bioavailability, potentially masking the natural progression of the disease in the data were not treated as separate groups, single-center study, small sample, no validation cohort, TBAS not specific for MDA, GGT is also a marker of hepatobiliary disease; we excluded known liver disease but did not perform liver imaging to exclude NAFLD.

Conclusion

The findings of this study suggest that elevated Vascular Uncoupling Index/Redox Imbalance Score, may be more strongly associated with endothelial dysfunction and hypertension than GGT, MDA, and NO assessed discretely. Targeted adjunctive interventions decoupling the GGT-MDA-NO pro-oxidant nexus in hypertensives may rescue endothelial dysfunction. This serves as a promising hypothesis-generating rationale for future interventional studies focusing on oxidative stress modulation and endothelial renovation strategies when validated in an independent cohort.

Conflict of interest

The authors declare that they have no conflict of interest.

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