ONLINE FIRST

ORIGINAL ARTICLE

Glutathione and Oxidized Low-Density Lipoprotein as Biomarkers of Oxidative Stress in Essential Hypertension

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doi: 10.22442/jlumhs.2023.00994

ABSTRACT

OBJECTIVE: This study aimed to compare the serum levels of Glutathione and Oxidized Low-Density Lipoprotein in the early hypertensive and normotensive subjects and to evaluate the diagnostic accuracy of these biomarkers for detecting oxidative stress in essential hypertension.
METHODOLOGY: An analytical cross-sectional study was conducted at Civil (Dr. Ruth K.M. Pfau) Hospital Karachi from July 2020 to March 2021 after approval from the ethical committee. After informed consent, the serum oxidized low-density lipoprotein, and glutathione levels were measured with ELISA's help in 40 normotensive and 40 early hypertensive participants. The results were analyzed by SPSS 21, keeping a significant p-value at <0.05.
RESULTS: The median levels of Oxidized Low-Density Lipoprotein were higher in hypertensive than in normotensive (i.e. 31.47 ng/ml vs 15.27 ng/ml). The median levels of glutathione were also raised in hypertensive compared with normotensive (i.e. 15.69 ng/ml vs 4.46 ng/ml). The differences were significant (p-value <0.001). Both markers showed adequate diagnostic accuracy, sensitivity and specificity to detect oxidative stress in early hypertension.
CONCLUSION: Oxidized Low-Density Lipoproteins are raised in early hypertension, endorsing the detrimental role of oxidative stress, while the levels of glutathione are increased to compensate for, and combat increased oxidative stress in early hypertension. These biomarkers can be utilized clinically to discern the burden of oxidative stress in early hypertension.

KEYWORDS: Essential hypertension, Glutathione, Oxidized low-density lipoprotein, Oxidative stress, Reactive oxygen species

INTRODUCTION

Hypertension is defined by the American College of Cardiology and American Heart Association (ACC/AHA) as blood pressure that is greater than 130/80 mmHg[1]. Essential hypertension is a widespread, chronic, non-communicable condition linked to several complications of cerebral, cardiac, renal, and ocular systems and their related end-organ damage. It includes multifactorial pathologies like genetic, metabolic, environmental and behavioral elements. Increased sympathetic activity, salt sensitivity, disturbances in the Renin-Angiotensin-Aldosterone-System (RAAS), and excessive reactive oxygen species formation have also been linked to causative connections[2].

Oxidative stress is appreciated as a discrepancy between reactive oxygen species/pro-oxidant and anti-oxidative molecules. It has emerged as a novel offender actively contributing to hypertension and other clinical conditions[3]. Reactive oxygen species (ROS) refers to the oxygen-derived free radical and associated non-radicle compounds accountable for the potent and aggressive oxidative damage to the cell and its components[3]. The cellular antioxidant defense system balances these negative consequences. If this neutralization is impaired or defective, it will lead to oxidative injury[4]. Due to the ongoing process of cellular oxidation-reduction reactions in the body and resultant oxidative damage, numerous oxidative products of lipid, protein, carbohydrates and deoxyribonucleic acid (DNA) are generated from the cell and tissues[5]. As biological indicators of any activity, response, or intervention that can be measured and replicated, these chemicals can be used as biomarkers to evaluate oxidative damage[6].

Clinical studies have shown that oxidative stress exists in essential hypertension and suggested various mechanisms that promote and sustain hypertension by reducing nitric oxide, interrupting vasodilatation, boosting the production of vasoconstrictor substances, accelerating apoptosis, bizarre vascular changes, etc[7]. However, the precise and most clear mechanism still needs further elucidation. Despite the above, some studies contradict the association of hypertension with oxidative stress and suggest systemic and end-organ inflammation, oxidative stress, and endothelial changes are not entirely responsible for hypertension[8], hence further studies are needed.

The oxidative stress biomarkers such as oxidized Low-Density Lipoprotein (Ox-LDL) and Glutathione (GSH) were quantified in this study. The Ox-LDL is an oxidatively modified low-density-lipoprotein that has changed structure and intrinsic properties. Since the typical receptors are unable to recognize it as a result of these altered modifications, Ox-LDL becomes the target of abnormal scavenger receptors, which encourages additional changes that boost chemotaxis and pro-inflammatory reactions, promote the formation of foam cells, and ultimately aid in the development of atherosclerosis[9]. Cysteine, glutamic acid, and glycine comprise the tripeptide known as GSH. It is a thiol buffer with a low molecular weight that is water soluble and functions as an antioxidant[9].

Understanding the link between hypertension and oxidative stress indicators could help evaluate the condition since they are quantifiable and affordable. Their quantification can assist us in identifying laboratory markers/parameters of disease prediction, diagnosis, and severity in addition to routine blood pressure checks. Their analysis could be beneficial if we promptly diagnose the disease and add antioxidants and antihypertensive medications. Therefore, effective patient care can spare many people from dreadful complications and early deaths, ultimately benefiting humankind.

This study assessed the presence of oxidative stress in the early stages of hypertension and the diagnostic precision for recognizing it. These biomarkers are affordable, repeatable, and trustworthy; their measurement can aid in identifying oxidative stress laboratory markers and parameters.

METHODOLOGY

The study was analytical cross-sectional in design, while a non-probability purposive sampling technique was done to recruit 80 individuals, comprising 40 normotensives and 40 hypertensives who had not yet received any medication. The sample size was estimated by Open Epi using mean difference and standard deviation (SD), comparing group 1 mean as: 1.8021, SD 0.422 and group 2 mean as: 1.2813, SD 0.331, with power 80, and confidence interval 95%, the ratio of sample size as 1, with the most related reference which was 9 in each group[10]. After ethical and institutional board approval, the study conducted from July 2020 to March 2021 in the medical outpatient wing of the Civil (Dr. Ruth K.M. Pfau) hospital in Karachi. Volunteers of either gender with ages ranging from 20 to 60 chosen. Pregnant women, anyone with any type of acute or chronic illness, and people taking antioxidant, lipid-lowering, or antihypertensive medications were excluded from the study. The numerous facets of the research explained to the volunteers, who then provided their signed approval. Primary demographic data was provided via a questionnaire. A general and physical evaluation was performed. The brachial artery blood pressure was measured with an electrical sphygmomanometer, with a cuff of 16 × 30 cm (for adults with arm circumference of 27 to 34 cm) and a cuff of 16 × 36 cm (for large adults with arm circumference of 35 to 44 cm). The recording was measured in a comfortable sitting posture after 10-15 min rest. The BP was taken at the time of participation and on the day of blood sampling. Also, B.P's records of the preceding days were checked. The average of the three measurements was taken.

About 4-5 cc of venous blood was collected under an aseptic measure, centrifuged at 2000 RPM for 10 min, and serum was used to detect the desired biomarkers with Sandwich Enzyme-linked immune sorbent assay, according to the provided manual instruction in duplication. The optical density (OD value) was identified with the help of a plate reader, kept at 450 nm.

Data analysis was performed by the statistical packages for the social sciences (SPSS-21). Non-parametric tests were applied. The Mann-U Whitney's test (Hypertensive/Normotensive) was used to determine associations and differences between the variables. Univariate and multivariable logistic regression analysis was used to estimate the Odds ratio (OR) with a 95% confidence interval (CI) of the hypertensive versus normotensive as the dependent variable for the cut-off values of Ox-LDL and Glutathione with optimal sensitivity and specificity, driven from the ROC curve and other possible confounders as independent variables. Spearman's correlation was applied to determine the relationships between the continuous variables. A p-value of <0.05 was considered statistically significant.

RESULTS

The study included 38 (47.5%) males and 42 (52.5%) females. The mean age of participants was 37.53±9.96 years. The mean systolic blood pressure (SBP) was 124.83±15.96 mmHg, and the mean diastolic blood pressure (DBP) was 80.20±13.13 mmHg. The average BMI was found to be 27.23±5.90 kg/m^2. Smoking was observed in 12 (15%) participants, 7 (58.3%) of which were normotensive.

Table I shows the biometric and biomarker comparison between hypertensive and normotensive groups.

Table I: Biometric and Biomarker Comparison between Normotensive and Hypertensive

Variables	Normotensive (n=40) Median (IQR, 25%-75%)	Hypertensive (n=40) Median (IQR, 25%-75%)	p-value
Age (years)	32 (6, 29-35)	40 (14, 36-50)	<0.001
Systolic BP (mmHg)	110 (12, 108-120)	138 (15, 130-145)	<0.001
Diastolic BP (mmHg)	70 (14, 61-75)	89.5 (15, 85-100)	<0.001
Height (cm)	163 (12, 158-170)	156 (8, 152-160)	<0.001
Weight (kg)	64 (14.5, 55.5-70)	75 (16, 68-84)	<0.001
BMI (kg/m^2)	22.45 (6.4, 20.5-26.9)	30.8 (6.8, 27.3 34.1)	<0.001
Ox-LDL (ng/ml)	15.27 (6.3, 12.4-18.7)	31.47 (13.6, 25.7-39.3)	<0.001
Glutathione (ng/ml)	4.46 (6.3, 1.3-7.6)	15.69 (11.5, 8.3-19.8)	<0.001

p-value calculated by the Mann-Whitney U test

Table II depict the correlation matrix of different parameters.

Table II: Spearman's Correlation analysis among different quantitative characteristics

		Systolic-BP (mmHg)	Diastolic-BP (mmHg)	BMI (kg/m^2)	Ox-LDL (ng/ml)	GSH (ng/ml)
Age (years)	r	0.52	0.48	0.38	0.44	0.25
	p-value	<0.001	<0.001	<0.001	<0.001	0.026
Systolic-BP (mmHg)	r	.	0.89	0.57	0.72	0.61
	p-value		<0.001	<0.001	<0.001	<0.001
Diastolic-BP (mmHg)	r		.	0.48	0.69	0.62
	p-value			<0.001	<0.001	<0.001
BMI (kg/m^2)	r			.	0.56	0.49
	p-value				<0.001	<0.001
Ox-LDL (ng/ml)	r				.	0.715
	p-value					<0.001

r represents the correlation value and p-value calculated using Spearman's correlation test.

Table III shows the univariate and multivariable analysis to estimate the factors associated with hypertension

Table III: Univariate and Multivariable Analysis to estimate the factors associated with hypertension

			Crude Odd Ratio (95% CI)	p-value	Adjusted Odd Ratio (95% CI)	p-value
Age (years)	20-30		1		1	
	31-40		3.15 (0.87-11.33)	0.079	8.20 (0.81-83.22)	0.075
	41-50		9.62 (1.95-47.44)	0.005	172.55 (2.05-14503.23)	0.023
	51-60		12.25 (1.78-83.94)	0.011	16.05 (0.51-505.42)	0.115
Gender	Male		1		1	0.493
	Female		1.83 (0.75-4.45)	0.181	2.24 (0.22-22.47)	
Smoking status	No		1		1	0.882
	Yes		0.673 (0.19-2.33)	0.533	1.32 (0.03-51.42)	
Ox-LDL (ng/ml)	Negative	≤20.61	1	0.000	1	0.001*
	Positive	>20.61	89.57 (17.38-461.39)		93.15 (5.90-1470.03)	
Glutathione (ng/ml)	Negative	≤ 5.94	1	0.000	1	0.008*
	Positive	> 5.94	91.00 (11.17-740.87)		40.32 (2.60-623.31)	

Univariate and multivariate logistic regression was applied for crude and adjusted odd ratios.

Table IV shows the oxidative biomarkers' diagnostic accuracy, sensitivity and specificity in detecting oxidative stress in hypertension. In the Receiver operating characteristic (ROC) curve, the values corresponding to the maximum diagnostic accuracy were 20.61 ng/ ml for the Ox-LDL and 5.94 ng/ ml for GSH.

Table IV: Diagnostic values of biomarkers in hypertensive patients

Parameters	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)	AUC
Ox-LDL (ng/ml)	95.0	82.5	84.4	94.2	88.7	0.962
Glutathione (ng/ml)	97.5	70.0	76.5	96.5	83.7	0.923

PPV indicates positive predictive value; NPV indicates negative predictive value, and the AUC area under the ROC

DISCUSSION

The raised levels of Ox-LDL in our study are according to studies which have elaborated it as the main culprit that accumulates in the vessel wall and enhances changes that lead to decreased arterial elasticity leading toward the gradual development of hypertension[11,12]. Increased Ox-LDL levels were also found in studies[13,14]. A detailed review by Maiolino G et al.[15] has reported studies that negate the association of Ox-LDL with cardiovascular-related diseases. A study by Kuklinska AM et al. [16] did not find significant differences in the hypertensive and normotensives.

Contrary to our analysis, most previously published investigations have shown that essential hypertension is associated with lower levels of GSH. In untreated mild to moderate hypertensive, erythrocyte GSH levels were found to be lowered[17,18]. While increased GSH levels in this study are comparable to those in studies by Razzaq A 2018[19] and Rybka J et al.[20]

Compared to healthy normotensives, early hypertensive had higher serum levels of glutathione and oxidized low-density lipoprotein, which strongly suggests an association between early hypertension and oxidative stress-related changes[21].

Researchers are studying numerous biochemical markers with etiology-specific predictive and diagnostic capabilities. Similarly to this, researchers are investigating the biomarkers of oxidative stress for several diseases, including hypertension[22]. Antioxidants can help blood pressure to some extent when used in conjunction with antihypertensive therapy, and studies have been conducted to demonstrate this link or causative relationship with hypertension. The increased glutathione levels in the current study could be explained by theories that have shown that GSH is poorly utilized at the cellular level when other associated enzymes are inefficient, by an accommodating response to oxidative stress that results in the induction of more glutathione molecules, or by the de novo synthesis of GSH that results from recycling of GSSG[20].

The other possible reason can be early hypertensive and young people with mild to moderate levels of oxidative stress that do not decrease but instead increase the levels as compensation to combat oxidative stress. Also, we have taken levels from serum, not the cell/ erythrocyte itself, which could be a possible reason for this difference.

Compared to previously published research[15], indicated a sensitivity of 67%, specificity of 66%, and AUC of 0.566. It was discovered that Ox-LDL in our study had greater diagnostic accuracy in identifying oxidative stress in hypertension patients. We also found that GSH had high diagnostic accuracy. Still, we could not locate any other studies that had measured diagnostic values of GSH in identifying oxidative stress in hypertensive patients. As a result, we can say that this study is novel because it used these specific biomarkers to assess oxidative stress in early hypertension and derive diagnostic accuracy values that can be considered for future clinical applications.

The present study's limitations include using only two biomarkers, a small sample size, and a cross-sectional methodology. While the short study period, the use of serum as a sample, and our use of the ELISA approach, where we discovered elevated glutathione levels in hypertension as opposed to other studies, are its strengths, they open up new avenues for further analysis. Additional research using additional biomarkers should be conducted in the early hypertensive groups, particularly in our nation, where hereditary, environmental, and lifestyle factors each play a distinct role.

CONCLUSION

According to the findings of this study, oxidative stress plays a significant role in the etiology of early hypertension, and biomarkers like glutathione and oxidized low-density lipoprotein can be used as reliable indicators of oxidative stress in this condition.

Acknowledgements: We acknowledge the study participants of Dow University of Health Sciences.

Ethical permission: Dow University of Health Sciences Institutional review board Letter No. IRB-1715/DUHS/Approval/2020.

Disclaimer: This study is a part of M.Phil. Physiology Thesis
Conflict of Interest: The authors have no conflict of interest to declare.
Financial Disclosure / Grant Approval: It was a self-funded study.

Data Sharing Statement: The corresponding author can provide the data proving the findings of this study on request. Privacy or ethical restrictions bound us from sharing the data publically.

AUTHOR CONTRIBUTIONS

Sahito FS: Concept, design of the work, acquisition of data and writing
Imtiaz F: Concept of the work and final approval of the manuscript
Qamar A: Concept of the work and final approval of the manuscript
Bhatty S: Concept of the work and final approval of the manuscript
Waseem HF: Data analysis and interpretation

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