Original Research Article

Evaluation of MMP-9 with atherogenic index and electrocardiogram as a predictor of cardiovascular risk factors in hypertensive patients

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ABSTRACT

Background: Hypertension is the strongest or one of the strongest risk factors for almost all different cardiovascular diseases acquired during life, including coronary disease, left ventricular hypertrophy, and valvular heart diseases. So, the aim of this study was to evaluate the plasma concentration of MMP-9 and AIP in mild to moderate hypertensive patients.

Materials and Methods: A total of 162 patients with hypertensive case were recruited and compared with the 100 normotensive controls from the Rohilkhand Medical College and Hospital Bareilly. Parameters include waist circumference (WC), Fasting Blood Sugar (FBS), Lipid profile with Atherogenic Index, and Matrix metalloproteinase-2 were estimated in the plasma of all the test groups. Electrocardiography (ECG) was also performed as a cardiovascular tool.

Result: There was a significantly increased concentration of WC, FBS, and Lipid parameters except for HDL which is slightly lower in the case group when compared to the control group. The concentration of MMP-9 was also increased, which is positively significant. The mean value of the P-R interval, QRS complex, and QT interval was also increased in hypertensive as compared to the controls.

Conclusion: Atherogenic index and MMP-9 are significantly higher in hypertensive patients. So, it could be a biomarker of cardiovascular disease.

1. Introduction

Major modifiable risk factors for cardiovascular disease include hypertension, smoking, diabetes mellitus, and abnormal lipid levels. The strongest evidence for causation is connected with hypertension, which has a high exposure prevalence worldwide.[1] 37% of all strokes and 18% of myocardial infarction (MI) cases globally are attributed to hypertension.[2] When either systolic, diastolic, or both are elevated, hypertension can be identified. Systolic blood pressure, which is higher when the heart is contracting (systole, and diastolic blood pressure, which is lower when the heart is relaxed (diastole).[3]

The majority of people (>95%) with high blood pressure have no known cause of their hypertension, which is sometimes referred to as primary or essential hypertension. The remaining 5% of patients had secondary causes found. The burden of hypertension disease is exacerbated by the presence of additional risk factors, such as dyslipidemia, which is more common in hypertensives and raises overall CV risk and results in atherosclerotic CV events.[3]

The extracellular matrix (ECM), which contains proteins like elastin and collagen fibers, gets rearranged in the vessels as a result of hypertension. In the extracellular

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matrix (ECM), the basement membrane, vascular smooth muscle cells migration, proliferation, contraction, and other processes, matrix metalloproteinases, which are proteolytic enzymes, can break down a variety of protein substrates. [4]

It has a specialized function in the development of blood vessels, tissue remodeling, and angiogenesis, all of which are triggered by a number of elements, including oxidative stress, inflammation, angiotensin II, and hemodynamic forces. [4] Chronically high blood pressure values can trigger the activation of all these mechanisms. Several MMPs have been studied in humans, but MMP-2 and MMP-9, also known as gelatinase A and gelatinase B, respectively, are particularly important for vascular remodeling and are also in charge of breaking down denatured collagen, elastin, fibronectin, laminin, and type IV and V collagens, which are primarily found in the endothelial basement membrane of the vessel wall. [5] Degradation of IV-type collagen, remodeling of the vasculature, angiogenesis, inflammation, and rupture of atherosclerotic plaque are all caused by MMP-2 and MMP-9. [4] The degradation of arteries' elastic lamina and the development of aneurysms in both people and animals are linked to elevated MMP-9 activity. The development of hypertension may be accelerated by any change in MMP-9. Arteries become stiff due to chronic hypertension. [6]

MMPs may break down a wide range of ECM constituents, and it has been suggested that unbalanced MMP activity is a key factor in the remodeling of hypertensive vascular structures. [6]

Because increased vascular stiffness and elevated pulse pressure were observed in hypertensive MMP-9 knock-out mice, a recent study hypothesized that MMP-9 plays a positive role early in angiotensin-II-induced hypertension. [6] Therefore, the primary goal of this study was to evaluate the growing cardiovascular risk in hypertension individuals.

2. Materials and Methods

2.1. Study design

Cross-sectional study. Standard normal range was used as control values for all parameters.

2.2. Study place

Department of Biochemistry, Rohilkhand Medical College & Hospital Bareilly (U.P).

2.3. Study duration

From September 2021 to March 2022.

2.4. Study subjects

A total of 162 patients with recently diagnosed mild-to-moderate hypertension with the age group of 18-44 years both male and female were compared with the 100 healthy individuals, who served as the study's controls visiting Medicine OPD, Rohilkhand Medical College & Hospital Bareilly.

2.5. Inclusion criteria

Patients with $\leq$ 130mmHg systolic blood pressure and $\leq$ 85mmHg diastolic blood pressure visit medicine OPD.

2.6. Exclusion criteria

1. Persons having a previous history of diabetes mellitus, cardiovascular disease, and taking any medicine to affect reduced Blood pressure and diabetes mellites.
2. Any renal, thyroid, and liver diseases are also excluded from the study.
3. Pregnant women or having last 2-year history of pregnancy were also excluded from the study.
4. Postmenopausal women and smokers & alcoholics were excluded from this study.

2.7. Study tools

1. Anthropometric measurements- Blood pressure, Waist circumference.
2. Laboratory investigations- lipid profile and MMP-9.
3. Another parameter is electrocardiogram.

2.8. Methods of assay

1. **Blood pressure measurement**: Blood pressure was measured by an auscultatory method using a standard cuff mercury sphygmomanometer on the left arm in a sitting position, after 5-10 min rest. [7]
2. **Waist circumferences :** The WC was measured at the midway point between the lower rib margin and the crest of the ileum in a horizontal plane at a standing position (cm). [8]
3. **Estimation of fasting blood sugar:** Fasting blood glucose level was determined by using the GOD-POD method (Glucose oxidase peroxidase method) by using of semi-autoanalyzer. [9]
4. **Estimation of serum lipid profile:** The following parameters were estimated by using a fully autoanalyzer using kits. [10]

   (a) Total cholesterol (TC), was estimated by using the CHOD-POD method.
   (b) Triglycerides (TG) were estimated by the GPO-PAP method.
   (c) High-Density lipoprotein-cholesterol (HDL-C) was estimated by CHOD-POD/Phosphotungstate method.
   (d) Low-density lipoprotein-cholesterol (LDL-C) was estimated by using FRIEDEWALD'S equation.
   (e) Atherogenic index was calculated by using the formula = log (TG/HDL-C). [11]

5. **Estimation of MMP-9 level** – The serum MMP-9 level was measured by Enzyme-linked immunosorbent assay (ELISA) using the kit. (Cat. No E0936Hu, BioAssay Technology, Shanghai Korain Biotech, China).[12]

6. **Electrocardiography (ECG) measurements:** Resting electrocardiography using a Sunfox Spandan 4.0 portable 12- lead ECG device was done. After explaining the procedure to the subject to allay anxiety, the upper clothing and all accessories (neckless, watches, rings) were removed. The electrocardiograph, which also consists of electrodes placed on the surface of the body, is used to perform the painless and straightforward ECG examination. The electrical currents are collected by the electrodes. The calibrations were 1mV=10 mm on the vertical line and an ECG speed of 25 mm/sec was used.[13]

This study was ethically approved (BIU/REG/Ph.D./321) by the institutional ethical committee of Rohilkhand Medical College. This research was conducted with Helsinki guidelines and informed consent was obtained from all participants.

2.9. Statistical analysis

All the parameters were expressed in Mean±SD. The independent t-test was applied among the various parameters for differentiating between the groups including the case and control groups. For testing qualitative data, the non-parametric chi-square test was used. The p-value <0.05 was considered statistically significant. All the statistical analysis was performed by means of IBM-SPSS (Statistical package for social sciences) version 23.0.

3. Results

A total of 162 hypertensive participants were selected for this study and compared with the 100 normal healthy controls. The demographic and clinical characteristics of both groups were summarized in Table 1.

The WC was increased and a p-value was <0.001, which is significant in the hypertensive group as compared to controls. Fasting blood glucose levels also increased in hypertensive subjects compared to the controls. A significant p-value (<0.001) was obtained. Lipid Profile, TC, TG, LDL, and VLDL were increased in hypertensive groups when compared to the controls which are significant, whereas HDL reduces in the hypertensive group as compared to controls. The enzymatic characterization MMP-9 level was higher in a hypertensive group than in control subjects. (Table 1). The mean of the P-R interval is higher in the hypertensive group when compared to the controls. As the same, the mean of the QRS and QT interval also increases and is positively significant in hypertensive patients as compared to the healthy controls. (Table 2).

The subdivision of the case group into prehypertensive and hypertensive groups. We found 16 abnormal ECG and 61 abnormal Atherogenic Index in 86 prehypertensive groups while in 76 hypertensive groups we get 31 abnormal ECG and 57 abnormal atherogenic indexes. (Table 3)

4. Discussion

Hypertension is recognized globally as a significant risk factor for CVD, stroke, diabetes, and renal diseases. About 80% of hypertensive persons have comorbidities such as obesity, glucose intolerance, and abnormalities in lipid metabolism, among others.[14] This study examined and evaluated the lipid abnormalities, atherogenic indexes, enzymatic abnormality, and ECG parameters among hypertensive patients as predictors of risk of developing cardiovascular diseases (CVDs).Diabetes one of the most common lifestyle disorders has been found to be associated with hypertensive patients. Various research has indicated this point very clearly since patients with hypertension often exhibit insulin resistance which might be the cause of developing diabetes in a later stage as compared to normotensive.[15] This study is not an exception as their hypertensive patients were presented with increased concentration of fasting blood sugar compared to normal healthy adults which have supported by a Korean-based study when they investigated that subjects with hypertension were at higher risk of diabetes than normotensive subjects and further suggested early blood pressure management since active blood pressure control reduced incident diabetes.[16]

In this study, the mean value of serum TC, TG, LDL, and VLDL concentrations are significantly higher in hypertensive patients than in normotensive controls. While the mean HDL level was lower in the hypertensives compared to normotensives and was statistically significant. Our findings of increased levels of these parameters in hypertensive patients are similar to the findings of some other studies.[11,14,17,18]

Electrocardiography is a non-invasive diagnostic modality that has a substantial clinical impact on investigating the severity of cardiovascular diseases. Eyoboglu M et al. concluded that fQRS may be a sign of increased blood pressure and may predict a higher fibrotic burden in patients with hypertension.[19] In this study, there was an increased QRS interval in hypertensive patients compared to normotensive patients. PR prolongation could be considered a marker for cardiovascular degenerative aging caused by vascular inflammation and myocardial fibrosis when Papakonstantinou, et al concluded that PR interval is a significant predictor of new-onset AF in hypertensive patients.[20] Similar to this there was a significantly increased PR interval in hypertensive patients compared to normal individuals.[21] QT interval is a measure of the duration of ventricular

Table 1: Difference among various parameters between case group and control group

Parameters	Hypertensive (162)	Control (100)	P value
WC	95.16±1.13	81.85±0.75	0.000
FBS	123.94±3.39	103.60±0.66	0.000
TC	201.37±2.421	173.15±2.25	0.041
TG	148.33±3.11	122.12±1.99	0.000
HDL	53.96±0.50	56.01±0.48	0.001
LDL	118.61±1.53	95.77±2.36	0.016
VLDL	29.88±0.62	24.32±0.39	0.000
TG/HDL	0.42±0.01	0.33±0.00	0.000
LDL/VLDL	2.89±0.04	1.82±0.05	0.001
MMP-9	121.65±1.74	50.51±0.49	0.001

Data were expressed as Mean±SD. P value<0.05 is considered statistically significant.

Table 2: Comparison of quantitative ECG parameters between hypertensive and controls

Quantitative ECG Parameters	Hypertensive (162)	Control (100)	P value
Mean PR interval (sec)	158.40±4.90	144.31±2.31	0.010
Mean QRS interval (sec)	106.5±2.11	85.23±1.80	0.000
Mean QT interval (sec)	357.01±8.37	382.02±5.37	0.010

Data were expressed as Mean±SD. P value<0.05 is considered statistically significant.

Table 3: ECG changes and Atherogenic index in different grades of hypertensive patients

Grading of Hypertension	Total 162	Electrocardiograph Normal	Abnormal	Chi-square	p-value	Atherogenic Index Normal	Abnormal	Chi-square	p-value
Prehypertensive	86	70	16	9.64	0.001	29	57	3.98	0.04
Hypertensive	76	45	31			15	61		

depolarization and repolarization. In this study, there was a significantly increased QT interval observed in hypertensive patients, which has also been found in myocardial infarction, hypertrophic cardiomyopathy, hypertension, left ventricular hypertrophy, diabetes, chronic heart failure, mitral valve prolapse Studies have reported that prolonged QT interval has been reported with increased cardiovascular death among subjects with diabetes, hypertrophic cardiomyopathy, and cardiac failure.[13,22,23] We observed that MMP-9 is present in significantly higher levels of hypertension than in normotensive controls which is consistent with previous studies.[24–26] While according to Wiktor Kuliczkowski's study there are no significant changes in mmp-9 concentrations in hypertensive.[27] Elevation of MMP-9 is important because abnormal MMP levels can stimulate vascular inflammation, a potential contributor to the pathogenesis and progression of hypertension.[28] Studies in the past have exposed the overproduction of MMP-9 plays a crucial role in the pathogenesis of coronary artery disease.[29] In support of this study, Friese R et al also observed similar findings when they observed the higher concentration of MMP-9 in hypertensive patients compared to normotensive control.[25] In this study there elevated concentration of MMP-9 was observed in hypertensive patients which might be responsible for the development of cardiovascular disease. In addition to this, studies have proven that deletion or inhibition of MMP-9 has proven beneficial in multiple animal models of cardiovascular disease.[28]

5. Conclusion

This study concludes that hypertension in the early stage is as harmful as in later stages, so proper blood pressure management requires as it is diagnosed. Although ECG findings cannot conclude an actual condition but can provide an actual direction in the general practice if taken into consideration. Abnormal findings of lipid profile with support of atherogenic index may define the presence of cardiovascular disease development in hypertensive patients. Increased concentration of MMP-9 in hypertensive may predict the presence of inflammation and fibrosis in cardiovascular disease. So, therapy related to reducing MMP-9 levels might be helpful to reduce the risk of cardiovascular disease. Further, studies with other parameters and an increased sample size might be conducted to explain a better picture of hypertension in the early stages.

6. Source of Funding

7. Conflict of Interest

None.

References

1. Campbell NRC, Whelton PK, Orias M, Wainford RD, Cappuccio FP, Ide N, et al. 2022 World Hypertension League, Resolve to Save Lives and International Society of Hypertension dietary sodium (salt) global call to action. *J Hum Hypertens*. 2022;17:1–10. doi:10.1038/s41371-022-00690-0.
2. DaSilva PM, Lima MJ, Neves P, Macedo MED. Prevalence of cardiovascular risk factors and other comorbidities in patients with hypertension in Portuguese primary health care populations: The PRECISE study. *Rev Port Cardiol (Engl Ed)*. 2019;38(6):427–37.
3. Hypertension: management. *Pharm J*. 2015;7(1). doi:10.1211/PJ.2015.20067719.
4. Hopps E, Presti RL, Caimi G. Matrix Metalloproteases in Arterial Hypertension and their Trend after Antihypertensive Treatment. *Kidney Blood Press Res*. 2017;42:347–57.
5. Yasmin, Mceniery CM, Wallace S, Dakham Z, Pulsalkar P, Maki-Petaja K, et al. Matrix metalloproteinase-9 (MMP-9), MMP-2, and serum elastase activity are associated with systolic hypertension and arterial stiffness. *Arterioscler Thromb Vasc Biol*. 2005;25(2):372.
6. Castro MM, Tanus-Santos JE, Gerlach RF. Matrix metalloproteinases: targets for doxycycline to prevent the vascular alterations of hypertension. *Pharmacol Res*. 2011;64(6):567–72.
7. James GD, Gerber LM. Measuring arterial blood pressure in humans: Auscultatory and automatic measurement techniques for human biological field studies. *Am J Hum Biol*. 2018;30(1). doi:10.1002/ajhb.23063.
8. Krupp K, Adsul P, Wilcox ML, Srinivas V, Frank E, Srinivas A, et al. Prevalence and correlates of metabolic syndrome among rural women in Mysore, India. *Indian Heart J*. 2020;72(6):582–58.
9. Ahmad A, Srikantiah RM, Yadav C, Agarwal A, Manjrekar PA, Hegde A. Cord Blood Insulin Levels: It's Correlation with Gender, Birth Weight and Placental Weight in Term Newborns. *Indian J Clin Biochem*. 2016;31(4):458–62.
10. Chahal J, Gupta S, Chawla SPS, Grewal H. Comparative study on fasting and postprandial lipid profile in type 2 diabetes mellitus. *J Family Med Prim Care*. 2021;10(3):1288–93.
11. Olamoyegun MA, Oluyombo R, Asaolu SO. Evaluation of dyslipidemia, lipid ratios, and atherogenic index as cardiovascular risk factors among semi-urban dwellers in Nigeria. *Ann Afr Med*. 2016;15(4):194–9.
12. Ahmad S, Singh V, Sinha RJ, Srivastava A, Mandhani A. Role of MMP-2, MMP-9 and VEGF as serum biomarker in early prognosis of renal cell carcinoma. *Afr J Urol*. 2018;24:255–63.
13. Akintunde AA, Oyedeji AT, Familoni OB, Ayodele OE, Opadijo OG. QT Interval prolongation and dispersion: Epidemiology and clinical correlates in subjects with newly diagnosed systemic hypertension in Nigeria. *J Cardiovasc Dis Res*. 2012;3(4):290–5.
14. Choudhury KN, Mainuddin AK, Wahiduzzaman M, Islam SM. Serum lipid profile and its association with hypertension in Bangladesh. *Vasc Health Risk Manag*. 2014;10:327–32.
15. Petrie JR, Guzik TJ, Touyz RM. Diabetes, Hypertension, and cardiovascular disease: Clinical Insights and Vascular Mechanisms. *Can J Cardiol*. 2018;34(5):575–84.
16. Kim MJ, Lim NK, Choi SJ, Park HY. Hypertension is an independent risk factor for type 2 diabetes: the Korean genome and epidemiology study. *Hypertens Res*. 2015;38(11):783–9.
17. Osuji CU, Omejua EG, Onwubuya EI, Ahaneku GI. Serum lipid profile of newly diagnosed hypertensive patients in nnewi, South-East Nigeria. *Int J Hypertens*. 2012;2012:710486. doi:10.1155/2012/710486.
18. Mahapatro AK, Chandrika S, Chepuru R. A Study of Lipid Profile Abnormalities among Patients with Essential Hypertension Attending Tertiary Care Centre. *Int J Contemporary Med Res*. 2022;7(1):1–4.
19. Eyuboglu M, Karabag Y, Karakoyun S, Senarslan O, Tanriverdi Z, Akdeniz B. Usefulness of fragmented QRS in hypertensive patients in the absence of left ventricular hypertrophy. *J Clin Hypertens (Greenwich)*. 2017;19(9):861–5.
20. Papakonstantinou P, Andrikou I, Konstantinidis D, Tsioufis K. Pr Interval Duration As A Risk Factor For New-Onset Atrial Fibrillation In Patients With Arterial Hypertension. *J Hypertens*. 2022;40(1):e70.
21. Vaidean GD, Manczuk M, Magnani JW. Atrial electrocardiography in obesity and hypertension: Clinical insights from the Polish-Norwegian Study (PONS). *Obesity (Silver Spring)*. 2016;24(12):2608–14.
22. Sun GZ, Zhou Y, Ye N, Wu SJ, Sun YX. Independent Influence of Blood Pressure on QTc Interval: Results from a General Chinese Population. *Biomed Res Int*. 2019;2019:1656123. doi:10.1155/2019/1656123.
23. Modak S, Mehta P, Patnaik J. Study on ECG Changes in Chronic hypertensive Patients in a Medical College Hospital of Odisha. *J Med Sci Clin Res*. 2016;4(5):10473–7.
24. Onal IK, Altun B, Onal ED, Kirkpantur A, Oz SG, Turgan C. Serum levels of MMP-9 and TIMP-1 in primary hypertension and effect of antihypertensive treatment. *Eur J Intern Med*. 2009;20(4):369–72.
25. Friese RS, Rao F, Khandrika S, Thomas B, Ziegler MG, Schmid-Schönbein GW, et al. Matrix metalloproteinases: discrete elevations in essential hypertension and hypertensive end-stage renal disease. *Clin Exp Hypertens*. 2009;31(7):521–33.
26. Valente FM, DeAndrade DO, Cosenso-Martin LN, Cesarino CB, Guimarães SM, Guimarães VB. Plasma levels of matrix metalloproteinase-9 are elevated in individuals with hypertensive crisis. *BMC Cardiovasc Disord*. 2020;20(1):132. doi:10.1186/s12872-020-01412-5.
27. Kuliczkowski W, Banaszkiewicz M, Mysiak A, Maka G, Bil-Lula I. Does Arterial Hypertension Affect Plasma Levels of Matrix Metalloproteinases and Their Tissue Inhibitors in Patients with Stable Coronary Artery Disease? A Preliminary Study. *Cardiol Res Pract*. 2019;2019:692131. doi:10.1155/2019/6921315.
28. Yabluchanskiy A, Ma Y, Iyer RP, Hall ME, Lindsey ML. Matrix metalloproteinase-9: Many shades of function in cardiovascular disease. *Physiology (Bethesda)*. 2013;28(6):391–403.
29. Hassanzadeh-Makoui R, Razi B, Aslani S, Imani D, Tabaee SS. The association between Matrix Metallo-proteinases-9 (MMP-9) gene family polymorphisms and risk of coronary artery disease (CAD): a systematic review and meta-analysis. *BMC Cardiovasc Disord*. 2020;20(1):232.

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