Title: D-dimer an independent risk factor for cardiovascular disease in hypertensive patients

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Grant Received: This work was carried out with the help of a research grant received from JSSAHER, Mysuru.(JSSAHER/REG/RES/URG/54/2023-2024/11507)

Ethical Clearance: Institutional ethical clearance was obtained before the commencement of the study: JSS/MC/PG/91 2022-2023

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Abstract:

Hypertension is a major risk factor for developing cardiovascular disease. The incidence of which is increasing both in India and worldwide. Cardiovascular disease leads to morbidity and mortality. Thus, a biomarker that helps in predicting cardiovascular mortality is warranted. D-dimer, a fibrinolytic degradation product, is found to be elevated in hypertensives and hypertensives with cardiovascular disease compared to normotensives. Elevated D-dimer results in increased blood viscosity, leading to thrombosis. In this pilot study, it was noted that the increase in D-dimer levels in hypertension is highly significant and independent of age and gender. Increased D-dimer thus serves as a predisposing factor for cardiovascular disease. Statins are reported to decrease D-dimer levels by 15%, therefore, prescribing statins to hypertensives may prove to be beneficial in the prevention of cardiovascular morbidity and mortality.

Key words: D-dimer, Cardiovascular disease, Normotensives, Hypertension, Statins

List of Abbreviations used: CVD: Cardiovascular Disease, FEU: Fibrinogen Equivalent Units, HTN: Hypertension, NT: Normotensives

Introduction:

Hypertension refers to an elevation in both systolic and diastolic blood pressure above the normal reference range of 120/80 mm of Hg. The incidence of hypertension is on an increasing trend both globally and in India. Currently, the incidence of hypertension is in India is around 27.6%.It is expected to reach around 30%. [1,2] The absence of major symptoms and lack of awareness among public regarding normal reference range and its direct link with cardiovascular disease poses a serious problem[3]. Males are more at risk of developing hypertension and urban population is more at risk, though the gap is narrowing. Hypertension mainly affects the older population, and the incidence increases with age. The incidence of hypertension is on an increase in young Indian adults. A study on young Indian adults in south India projected that about 11.2% in the age group of 20 to 39 years had an elevated blood pressure. Hypertension. If not controlled and treated results in renal disease, stroke, and cardiovascular diseases[4]. Of these, cardiovascular disease is of major concern as it is leading to premature deaths in young adults. This results in a decreased workforce and is responsible for economic slowdown and poses a social burden. Hypertension is symptomless and hence is detected mostly during routine medical examination[5]. Thus, there is an urgent need for identifying a reliable marker for predicting cardiovascular events in hypertensive patients. Hypertension is also responsible for increasing blood viscosity, restricting blood flow, intravascular damage and thrombosis [6,7,8]

D-dimer is a soluble fibrinolytic product formed from the fibrinolytic degradation of clots, by the fibrinolytic system. Structurally 2 D domains and an E domain of the original fibrinogen molecule constitute the D-dimer molecule, having a half-life of about 6 to 8 hours. Elevated levels of circulating D-dimer pose an increased risk of thrombosis.

Pulmonary embolism, Deep Vein Thrombosis, and intravascular coagulation disorders are associated with increased D-dimer levels[9]. D-dimer level is a predictive marker for the diagnosis and prognosis of deep vein thrombosis. Inflammation followed by thrombosis results in the progression of atherosclerosis and coronary artery disease[10]. D-dimer levels are increased in myocardial infarction and is a predictor of future cardiovascular events in healthy individuals[11]. Severity of hypertension influences D-dimer levels. Studies show a direct relationship between severity of hypertension and D-dimer levels. The risk of thromboembolism increases with an increase in D-dimer levels. D-dimer levels are predictors of microvascular health and increased levels result in microvascular complications[12]. D-dimer levels are known to result in mortality irrespective of cardiac involvement and statins are shown to reduce D-dimer levels more effectively compared to antiplatelet drugs[13] .As the increased D-dimer level poses a high risk and may result in mortality estimation of D-dimer levels and steps to reduce D-dimer levels is necessary

An independent risk predictor is warranted as cardiovascular events precede chronic renal failure and stroke. Thus, we wanted to estimate the levels of D-dimer, a fibrinolytic product and coagulation marker in age and sex matched normotensives, hypertensives and hypertensives with cardiovascular disease.

Methodology:

A cross-sectional analytical study to assess the risk of cardiovascular disease in hyper tensive patients based on D-dimer levels was performed at JSS Hospital Mysuru. The study population included patients undergoing treatment at JSS Hospital, Mysuru, during January to May 2024.

Sampling: Convenient sampling methods were employed for this study. Initially, Patients admitted to cardiac Intensive care unit, with hypertension were selected and their D-dimer levels were estimated. These values were compared with age and sex matched hypertensive patients without cardiovascular disease and normotensive subjects. The sample size was calculated as follows: The Incidence of Hypertension in India is 24%. Based on this the sample size was calculated as 97 rounded of to 100, taking confidence interval of 80% and precision of 5%.

Inclusion Criteria:
Normotensive Subjects, Hypertensive patients without cardiovascular disease Hypertensive patients with cardiovascular disease in the age group of 30 to 80 years. The subjects were divided into 3 groups,
Group A: Non-Hypertensive subjects without any cardiovascular disease.
Group B: Hypertensive patients with cardiovascular disease.
Group C: Hypertensive patients without any cardiovascular disease.

Exclusion **Criteria:**
Patients diagnosed with deep vein Thrombosis, Pulmonary Embolism, and Intravascular thrombosis were excluded from the study.

Procedure:
After obtaining Ethical clearance and informed consent,2 ml of venous blood was drawn under aseptic conditions from the median cubital vein, using light blue topped vacutainers containing 3.2% sodium citrate. D-dimer levels were estimated by Latex Enhanced Immunoturbidimetry using Roche autoanalyzer.

Principle:
D-dimer particles in the sample bind to anti D-dimer antibodies coated on to immobilize latex particles. This results in agglutination and the resulting turbidity is measured spectrophotometrically at 570 nm.
The normal reference range of D-dimer is up to 0.50 mg/L of fibrinogen equivalent units (FEU).

Data Collection:

Data including demographic details, disease symptoms, laboratory investigations were obtained from patient files, and hospital information system of JSS Hospital, Mysuru. The following information was collected for each patient during their admission period: name, age, sex, history of HTN, and highest recorded D-dimer level.

Data Analysis:
The D-dimer levels of Normotensive controls were compared with

Hypertensive subjects and Hypertensive subjects. The chi-square test was used for statistical analysis. The chi-square test is a statistical analysis. that makes a comparison between expected and observed data. This helps establish the relationship between variables under consideration. The significance and correlation were analysed using Students T test and one-way ANOVA (Analysis of Variance)

Ethical Considerations This cross-sectional study was conducted after obtaining ethical clearance from the institutional ethical clearance committee of JSS Medical College (Mysuru. (reference: JSS/MC/PG/91/2022-23) dated31/03/2023.Samples were collected after informed consent from the concerned subjects participating in the study.

Results:

Demographic details of the subjects indicated that the highest percentage of subjects were in the age group of 31 to 40 years. The percentage is decreasing as the age increases. The incidence was found to be more in males compared to females. Sixty percent of the study subjects were males as compared to 40% of females. The age and gender-based demographics are depicted in Figure 1

Figure 1:Age and gender based demographics

D-dimer levels increase with an increase in Blood Pressure.

The study subjects were grouped into 3 groups and were age and sex matched. Normotensives, Hypertensives and patients who have cardiovascular disease in addition to hypertension.

D-dimer levels showed an increase in patients with cardiovascular disease , compared to normotensives. There was an increase in D-dimer levels in hypertensive subjects without cardiovascular disease compared to normotensives. D-dimer levels in patients with cardiovascular disease showed the greatest increase compared to both normotensive and hypertensive subjects without CVD. The results are depicted in Table 1.

Table 1:D Dimer levels in age and sex matched normotensives, Hypertensives and Hypertensives with CVD.

Age in Years	Gender	Normotensives	Hypertensives	Hypertensives with CVD
33	M	0.3	0.5	0.7
33	M	0.2	0.6	0.8
36	M	0.1	0.6	0.6
37	M	0.1	0.1	0.7
41	F	0.5	1.2	8.7
41	M	0.3	1	0.7
43	F	0.3	0.6	3.4
44	M	0.1	0.8	0.9
45	M	0.1	2.2	4.7
50	F	0.3	0.7	0.9
50	F	0.2	1.8	6.5
50	F	0.2	1.7	6.5
52	F	0.1	1.4	0.8
52	F	0.3	0.5	0.7
53	M	0.1	0.6	3.5
54	M	0.1	1	2.6
54	M	0.1	0.2	0.9
55	F	0.1	1.2	3.2
55	M	0.1	0.9	3
58	F	0.5	0.7	1.1
58	F	0.2	0.9	0.9
60	M	0.4	0.5	0.8
62	M	0.1	1.2	1.8
65	F	0.1	0.8	3.2
66	M	0.1	0.3	3
66	M	0.4	0.5	0.6
67	F	0.5	1.8	3.9
68	M	0.4	0.4	0.6
68	M	0.5	0.9	3.6
72	F	0.2	0.6	0.9
73	M	0.3	0.7	1
80	M	0.3	0.5	0.8
80	M	0.5	0.6	0.8

D-dimer levels are significantly higher in Hypertensive subjects compared to normotensive subjects. D-dimer levels were determined in 34 subjects. Only 2 of the 34 normotensives had high D-dimer levels as compared to 25 hypertensive subjects and 28 hypertensive subjects with cardiovascular disease. The result is highly significant (P value <0.001).The results are depicted in Table 2 and Figure 2.

Table 2:Comparison of D Dimer levels in normotensives, Hypertensives and Hypertensives with CVD

		D- Dimer Category			Total	Chi square value	p value
		High	Less	Normal			
HTN-CVD-C	CONTROL	2	26	5	33	51.803	<0.001
	HTN	25	4	3	32		
	HTN-CVD	28	4	2	34		
Total		55	34	10	99		

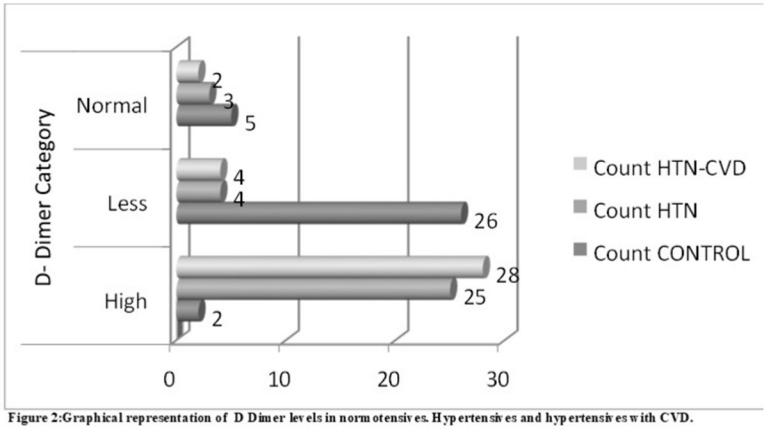

Figure 2:Graphical representation of D Dimer levels in normotensives. Hypertensives and hypertensives with CVD.

D-dimer levels in males increase with increase in blood pressure and cardiovascular disease:

D-dimer levels were also compared between normotensive men and hypertensive men. There was a significant increase in D-dimer levels in hypertensive men($p<0.005$).On comparison with hypertensive men with cardiovascular disease, the increase was more significant ($p<0.002$). Figure 3 is a graphical representation of D-dimer levels in male normotensives, hypertensives, and hypertensives with CVD.

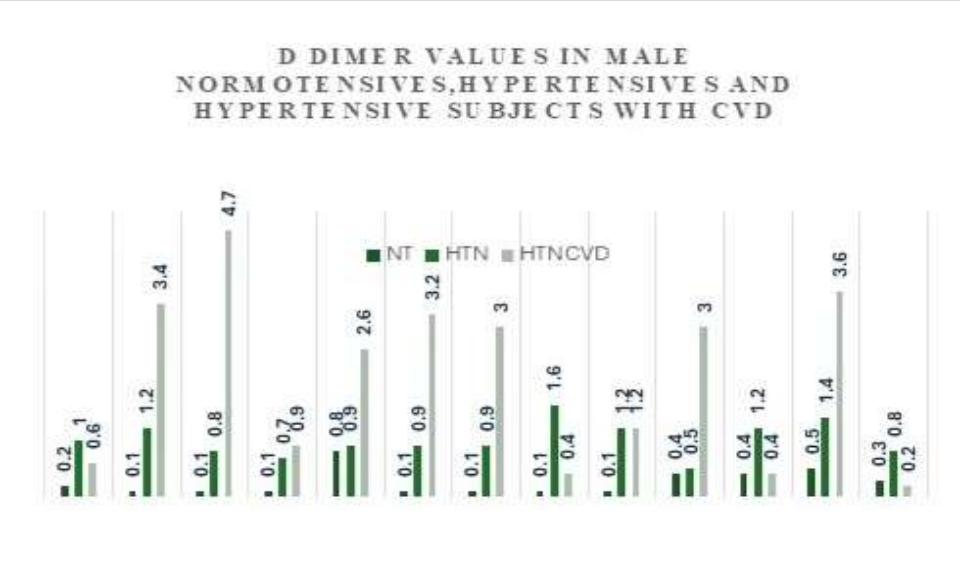

Figure 3:Graphical representation of D-dimer levels in normotensive, Hypertensive, and Hypertensive men with cardiovascular disease.

D-dimer levels in females increase with an increase in blood pressure and cardiovascular disease:

D-dimer levels were also compared between normotensive women and hypertensive women. There was a significant increase in D D-dimer levels in hypertensive women($p<0.05$).On comparison with hypertensive women with cardiovascular disease, the increase was highly significant ($p<0.01$). Figure 4 is a graphical representation of D-dimer levels in female normotensives, hypertensives, and hypertensives with CVD.

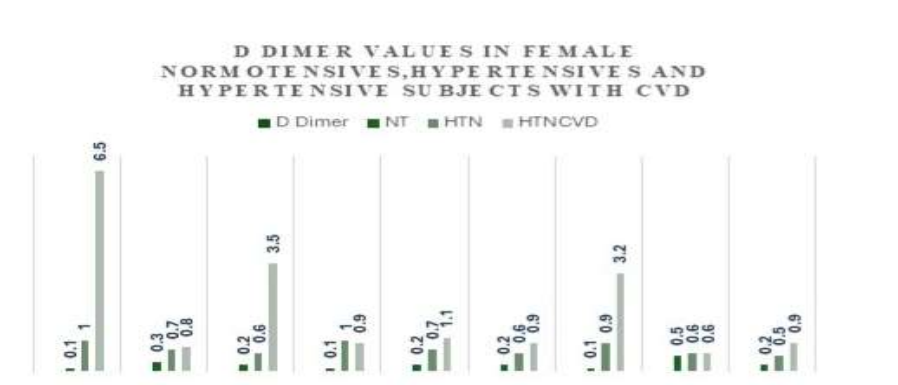

Figure 4:Graphical representation of D-dimer levels in normotensive, hypertensive, and hypertensive women with cardiovascular disease.

D-dimer levels were high in both men and women hypertensives with CVD($P<0.05$), compared to hypertensives without CVD.

D-dimer levels in males and females increase with an increase in hypertension independent of age:

D-dimer levels were compared with increasing age in normotensive, hypertensive, and hypertensive women with CVD. D-dimer levels were higher in hypertensive women compared to normotensives ($p < 0.005$). Higher values were also observed in hypertensive women with CVD compared to normotensives($p<.005$). Hypertensive women with cardiovascular disease had higher D-dimer levels compared to hypertensives without cardiovascular disease($p<.03$). This is depicted in Table 3.

Table3:D Dimer levels in male normotensives ,hypertensives and hypertensives with cardiovascular disease with respect to age:

Age in years	NT	HTN	HTNCVD
73	0.3	0.5	0.2
68	0.4	1.8	0.4
33	0.2	1.4	0.5
36	0.1	0.1	0.6
68	0.5	0.6	3.6
62	0.1	1.8	1.2
60	0.4	0.8	0.4
54	0.1	0.2	2.6
37	0.1	0.6	0.7
66	0.1	0.8	3
80	0.3	0.8	0.5
45	2.2	0.1	4.7
53	0.1	1.4	0.9
73	0.3	0.7	1
66	0.4	0.3	3.9
80	0.5	0.6	0.8
44	0.8	0.1	3.4
54	0.1	1.2	3.2
55	0.1	0.9	3
41	0.3	1.2	0.7

D-dimer levels were compared with increasing age in normotensive, hypertensive, and hypertensive men with CVD.D-dimer levels were higher in hypertensive men compared to normotensives ($p < 0.01$). Higher values were also observed in hypertensive men with CVD compared to normotensives($p<.0002$).Hypertensive men with cardiovascular disease had higher D-dimer levels compared to hypertensives without cardiovascular disease($p<.009$).This is depicted in table 4.

Table4:D Dimer levels in male normotensives ,hypertensives and hypertensives with cardiovascular disease with respect to age:

Age in years	NT	HTN	HTNCVD
41	0.5	1	8.7
43	0.3	0.6	0.9
50	0.2	1.7	6.5
50	0.2	1.8	6.5
50	0.3	0.7	0.8
52	0.1	0.5	0.7
52	0.3	0.6	3.5
55	0.1	1	0.9
58	0.5	0.7	1.1
58	0.2	0.9	0.9
65	0.1	0.5	3.2
67	0.5	0.9	0.6
72	0.2	0.6	0.9

Discussion:

Cardiovascular diseases are currently the leading cause of death globally. The major cause of death is myocardial infarction and stroke. The incidence of cardiovascular disease is on an increasing trend. Cardiovascular diseases were responsible for 20.5 million deaths globally, constituting about 30% of global deaths[14]. The major risk factors associated with cardiovascular disease are hypertension and Diabetes Mellitus. The Indian scenario is of great concern as the incidence is higher than the global average and occurs at an earlier age. The age-standardized CVD death rate in India is about 272 per one lakh population as compared to worldwide average of 235.The incidence at a younger age result in psychosocial problems and leads to an economic burden as the patient has to modify lifestyles and diet and problems encountered in returning to normal[15].

The most strategic risk factor for cardiovascular disease is hypertension. In the early stages, it is symptomless and often goes unnoticed. Lack of awareness among the public about the consequences of neglecting treatment is also responsible for mortality.

Hypertension is responsible for increasing the viscosity and coagulability of blood. It is also known to cause a change in the structure of platelets, resulting in a prothrombotic state[16]. D-dimer is a soluble degradation product of fibrins, and the levels are increased in thrombosis. D-dimer levels are increased in microvascular disease[17]. Hypertension is directly linked to the development of cardiovascular disease; thus, in this study we examined D-dimer levels to find whether there is any correlation between D-dimer levels, hypertension, and cardiovascular disease. Thirty normotensives, thirty hypertensives, and 30 hypertensives with CVD were included in the study, and D-dimer levels were estimated using latex-enhanced immunoturbidimetry. Higher D-dimer levels were observed in 28 out of 34 subjects with CVD and 26 out of 32 hypertensive patients. Two out of 32 normotensives had higher D-dimer levels. Thus, higher D-dimer levels are associated with an increase in blood pressure and cardiovascular disease. ($p<0.001$). Various studies have shown that D-dimer levels are associated with higher rates of mortality irrespective of cardiac involvement[18]. The use of statins has been shown to reduce D-dimer levels to a greater extent when compared to antiplatelet drugs[19].Antiplatelet drugs, did not show a potential decrease in D-dimer Levels[20].

To rule out the effects of gender bias, D-dimer levels were correlated between male and female normotensives and hypertensives. D-dimer levels were significantly increased in both male and female hypertensives compared to normotensives ($p<.005$).D-dimer levels were higher in both males and female hypertensives with cardiovascular disease($P<0.005$).D-dimer values were compared in normotensive and hypertensive males and females concerning age. D-dimer values were found to be significantly high in Hypertensive males and females, irrespective of age($p<0.01$ and $p<0.005$), respectively. D-dimer values were found to be highly significant in hypertensives with cardiovascular disease compared to hypertensives without cardiovascular disease, irrespective of age in both males and females($p<0.009$ and $p<0.03$), respectively. This pilot study shows a strong indication that D-dimer is an independent risk factor in the development of cardiovascular disease.

Conclusion: Steps to reduce D-dimer levels may prove beneficial in preventing the development of cardiovascular disease. As statins are known to decrease D-dimer levels by about 15%, It may be beneficial to prescribe statins to hypertensive patients to reduce their risk of developing cardiovascular disease. The limitation of this study is that it involved a limited number of participants, and a study with a larger sample size may yield more meaningful and generalizable results.

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