Evaluation of hypertension with or without obesity and its correlation with MMP-9 and TIMP-1 - A hospital based cross sectional study.

BY

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Dissertation submitted to

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I, Miss. Shahida H Byadagi with this declares that this dissertation entailed- **"Evaluation of hypertension with or without obesity and its correlation with MMP-9 and TIMP-1 - A hospital based cross sectional study"**. Is a bonafide and genuine research work carried out by me under the guidance of Dr. Basavaraj Devaranavadagi, Professor and Head ,Dept. of Biochemistry BLDE (Deemed to be University), Shri B.M.Patil medical college, Hospital & research centre Vijayapura.

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Shahida H Byadagi.

LIST OF ABBREVIATIONS

1. AH- Arterial hypertension
2. Ag II-Angiotensin II
3. ATP- Adenosine triphosphate
4. B- Blank
5. BMI- Body mass index
6. BP- Blood pressure
7. CVC- Cardiovascular complications
8. CV- Cardiovascular
9. CVD- Cardiovascular disease
10. D/W- Distilled water
11. DBP- Diastolic blood pressure
12. DNA - Deoxyribonucleic acid
13. DTNB- 5,5' Dithibis -2-Nitrobenzoic acid
14. ECM- Extracellular matrix
15. EDTA- Ethylene di-amine tetra acetic acid
16. ELISA- Enzyme-linked Immunosorbent assay
17. eNOS- Endothelium Nitric oxide synthase
18. ESC- European Society of Cardiology
19. ESH- European Society of Hypertension
20. g- Gram
21. GSH- Reduced glutathione
22. H2O2-Hydrogen peroxide
23. H2SO4- Sulphuric acid
24. HC- Hip circumference
25. HCl- Hydrochloric acid
26. HF- Heart failure
27. HRP- Horseradish peroxidase
28. HTN- Hypertension
29. JNC- Joint national committee
30. L- Litter
31. LPH- Lipid hydroperoxide
32. LPS- Lipofuscin
33. MDA- Malondialdehyde
34. ml- Milliliter
35. MMP- Matrix metalloproteinases
36. MUO- Metabolically unhealthy obese
37. Na2HPO4- Disodium hydrogen monophosphate
38. NaNO2- Sodium nitrite
39. NaOH- Sodium hydroxide
40. ng- Nanogram
41. nm- Nanometer
42. nmol- Nano mole
43. NO- Nitric oxide
44. NO2-Nitrogen dioxide
45. NO3-Nitrite
46. NOs- Nitric oxide synthase
47. O-2-Superoxide radical

48. OD- Optical density
49. OH- Hydroxyl radicals
50. OSI- Oxidative stress index
51. pH - Potentials of hydrogen
52. PUFA- Polyunsaturated fatty acid
53. RBC- Red blood cell
54. RNA- Ribo nuclic acid
55. ROS- Reactive oxygen species
56. RASS-renin-angiotensin system
57. rpm- Rotation per minute
58. SBP- Systolic blood pressure
59. SEA- Serum elastase activity.
60. SNS- Sympathetic nervous sysytem
61. SOD- Superoxide dismutases
62. S- Standard
63. TAC- Total antioxidant capacity
64. TBA- Thiobarbituric acid
65. TCA- Trichloroacetic acid
66. T- Test
67. TIMP- Tissue inhibitors of matrix metalloproteinases
68. TOS- Total oxidative stress
69. TNF-α- Tumor necrosis factor
70. UV- Ultra violate
71. VCl3-Vanadium chloride
72. VEGF-Vascular endothelial growth factor
73. VSMC- Vascular smooth muscle cells
74. WC-Waist circumference
75. WHO-world health organization
76. WHR- Waist-to-hip ratio
77. WHtR- Waist-to-height ratio
78. ZnSO4-Zinc sulfate
79. °C- Celsius
80. µL - Microlittere
81. µm- Micromol
82. % -Percentage
83. RASS-renin-angiotensin system

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ABSTRACT

Background and Purpose:

Hypertension is a significant risk factor for cardiovascular diseases associated with obesity. Matrix metalloproteinase-9 (MMP-9) and tissue inhibitor of metalloproteinase-1 (TIMP-1) are enzymes involved in vascular remodeling and may play a role in the pathogenesis of hypertension. This study aimed to evaluate serum MMP-9 and TIMP-1 levels in obese and non-obese hypertensive and non-hypertensive individuals and to investigate the correlation between anthropometric parameters, MMP-9 and TIMP-1, as well as the association between oxidative stress and MMP-9 & TIMP-1 in these individuals.

Methods:

A cross-sectional study was conducted on 106 subjects, including 53 normotensive and 53 hypertensive individuals, who were further divided into obese and non-obese subgroups. Serum levels of MMP-9 and TIMP-1 were measured using ELISA, while oxidative stress parameters (MDA, GSH, NO) were measured using manual methods. Statistical analysis was performed using a p-value of <0.05 as statistically significant.

Results:

All four groups showed significant differences in serum levels of MMP-9 and TIMP-1, with p-values <0.05. Positive correlations were observed between serum levels of TIMP-1 and MMP-9 and increased blood pressure. Furthermore, a positive correlation was observed between MMP-9 and MDA, while negative correlations were observed between MMP-9 and GSH and NO.

Conclusion:

Elevated serum levels of MMP-9 and oxidative stress parameters and decreased antioxidant enzyme levels may contribute to vascular remodeling and the development of hypertension. The increase in TIMP-1 and MMP-9 levels may indicate compensatory inhibition of extracellular matrix breakdown and collagen deposition leading to vascular fibrosis. The positive correlation between MMP-9 and MDA and negative correlations between MMP-9 and GSH and NO suggest a relationship between MMP-9 and oxidative stress. These findings suggest that MMP-9 and TIMP-1 may play a role in the pathogenesis of hypertension in obese and non-obese individuals.

Keywords: Hypertension, Obesity, MMP-9, TIMP-1, Oxidative stress

INTRODUCTION

As per Joint National Committee-8 (JNC 8) guidelines Systolic blood pressure (SBP) more than or equal to 140 mm Hg and diastolic blood pressure (DBP) more than or equal to 90 mm Hg constitutes hypertension (HTN). Because it persists asymptomatically for a longer time and harms the body severely, is known as a "**Silent killer.**" [1] Additionally, it raises the risk of kidney, cerebrovascular, and cardiovascular (CVD) conditions, including heart disease, heart failure, and stroke. As a result, the body experiences a variety of physiological and pathological alterations. Thus, HTN is a significant global source of illness and mortality. [2] Around 1.38 billion individuals worldwide suffer from hypertension (HTN). Nearly two-thirds of them are from low- and middle-income nations; based on Jamshed D et al. studies, HTN is 29.8% common in India, with rural areas having a prevalence of 27.6% and urban areas having a frequency of 33.8%. Lack of awareness, therapy, and control raises the risk of HTN. Controlling hypertension is crucial for lowering adverse severe cardiovascular consequences and deaths linked to them. [3]

1.1. CLASSIFICATION OF HTN:

The Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC-8) categorized hypertension into Stages 1 and 2 and Pre-hypertension in its eighth report, which also established norms for normal blood pressure.

I. Adult's blood pressure is categorized in accordance with the Joint National Committee (JNC 8).

	Systolic	**Diastolic**
Normal	Less than or equal to 120	And Less than or equal to 80
Pre-hypertension	120 to 139	Or 80 to 89
Stage-1 Hypertension	140 to 159	Or 90 to 99
Stage-2 Hypertension	More than or equal to 160	Or More than or equal to 100

1.2 HTN types and definition

1.2.1 Primary/essential HTN

1.2.2 Secondary HTN

1.2.1. Primary/essential HTN:

Essential hypertension is a rise in blood pressure with no known cause that increases the risk to the brain, renal, and cardiac systems. Commonly, essential hypertension occurs in conjunction with other cardiovascular risk factors like age, being overweight, insulin resistance, diabetes mellitus, and hyperlipidemia. [4]

1.2.2. Secondary HTN:

Secondary hypertension is a form of high blood pressure with an underlying, treatable cause. Reno vascular hypertension, primary aldosteronism, and parenchymal renal disease are the leading causes of secondary hypertension. [5]

1.3 PREVALENCE OF HYPERTENSION:

HTN is a severe global public health issue undergoing socioeconomic and epidemiological changes. [6] It is the most frequent, identifiable, and treatable CVD risk [7]. HTN affects 80% of people world-wide due to modern lifestyles and obesity in underdeveloped and developing nations. [1] Essential HTN affects around one billion individuals; by 2025, that number will rise to 1.58 billion. [7]

About 33% of Indians living in cities and 25% living in rural areas suffering from HTN. Only 25% of Indians in rural areas and 42% in urban areas know they have HTN. 38% of urbanite and 25% of rural residents are receiving treatment. Thus, just one-third of India's hypertensive rural and urban populations have their blood pressure under control. [7]

1.4 OBESITY AND HTN:

Regarding HTN, obesity has received much attention. Globally, obesity is an epidemic that is directly associated with metabolic syndrome or metabolic problems such as an increased waist circumference (WC), hip circumference (HP), blood pressure (BP), and dyslipidemia. Obesity, particularly the abnormal visceral fat distribution causes hormonal, inflammatory, and endothelial changes. These changes stimulated several processes that support HTN. On the other hand, it increased cardiovascular morbidity and mortality[49]

The body mass index (BMI) is a tool for measuring obesity. However, BMI alone should not be used to distinguish between lean muscle mass and body fat; instead, a combination of BMI and WC is a reliable indicator of disorders associated with obesity. Important early indicators of obesity include anthropometric measurements like BMI, WC, HC, WHR (waist-to-hip ratio), and WHtR (waist-to-height ratio). [1] Compared to a person with a high BMI and no abdominal obesity, the person with abdominal obesity with a normal BMI is more at risk for cardiovascular disease (CVD). [8]

II.BMI - based obesity classification according to the WHO [9]

Diagnosis	BMI (kg/m2)	The possibility of disease Male WC (cm) >94 Females ≤ 80	The possibility of disease Male WC (cm) > 94 Females > 80
Underweight	less than 18.5		
Normal weight	18.5–24.9		
Overweight	25 to 29.9	Increased	High
First-class Obesity	30 to 34.9	High	Very high
Second-class Obesity	35 to 39.9	very elevated	very elevated
Third-class obesity	more than or equal to 40	Extremely elevated	Extremely elevated

1.5 PATHOPHYSIOLOGY OF HYPERTENSION IN OBESITY:

The transformation of a normotensive phenotype into hypertension is mediated by a complex interaction of genetic, environmental, behavioral, and nutritional variables. Together, obesity and hypertension have two significant impacts. First, this combination is dangerous because it raises the risk of (CVD) in persons who are obese and have high blood pressure, including stroke, end-stage renal disease, congestive heart failure, sudden cardiac death, and coronary heart disease.[50] Second, obesity increases the likelihood of developing Hypertension of the vasculature resistant to treatment. This condition calls for a mix of medication and device therapy, including renal sympathetic denervation. On the other hand, population studies have revealed that weight increase in the future is noticeably more significant than in normotensive

individuals with hypertension. This data demonstrates how likely it is for people with hypertension to gain weight in the future and supports the idea that obesity and HTN are associated. [10]

1.6 Matrix Metalloproteinase in HTN

The Matrix Metalloproteinase (MMP) is a zinc-dependent protease that is accountable for the degeneration of several extracellular matrix (ECM) proteins like collagen and fibrinogen and vascular smooth muscle cells (VSMC)[53]. This might cause cell migration and growth. These outcomes lead to persistent vascular remodelling and dysfunction, which are linked to HTN maladaptive outcomes. However, elevated MMPs are also linked to intracellular effects in cardiomyocytes. They do not just damage ECM protein but also contribute to the maladaptive remodelling of the heart and vasculature.

The MMPs are classified according to ECM substrates as Collagenases, Stromelysins, matrilysin, and membrane-type and gelatinases families[51]. The MMP is a class of zinc-dependent proteases that destroys many extracellular matrix (ECM) proteins, including fibrinogen, collagen, and vascular smooth muscle cells (VSMC).[52] This could cause cell migration and proliferation. These effects lead to chronic vascular dysfunction and remodelling, linked to HTN-related maladaptive outcomes. However, the elevated levels of MMPs are also linked to the intracellular impacts in cardiomyocytes, breaking down ECM proteins, altering the contractile system and contributing to the adverse remodeling of the heart and vascular system.[11]

III. CLASSIFICATION OF MATRIX METALLOPROTEINASE ENZYME [12]

Traditional classification	**Numerical classification**	**Biological effect**	**Location on the chromosome**	**group of enzyme substrates**
MMP categorization				
Collagenases				
Collagenase-1	MMP-1	Movement of keratinocytes and other cells,platelet aggregation, a rise in insulin-like growth factor -1 bioavailability, tumor development, an impact that promotes inflammation, and activation of polymerase-1; the development of the cancer-proteolytic activity, the breakdown of physical barriers;	11q22-q23	Casein, entactin, laminin, pro-MMP-1, pro-MMP-2, and pro-MMP-9, as well as collagen types 1, 2,3, 4,5 and 10, as well as serpins

Collagenase-2	MMP-8	The induction of chemokine breakdown, increased affinity for collagen, production of- Fibroblast growth factor, activating osteoclasts, enhancement of anti-inflammatory action, and regulation of mobilization during tumorigenesis.	11q21-q22	Gelatin, aggrecan, fibronectin, collagen (1 to 3, 5, 7, 8, and 10), and others
Collagenase-3	MMP-13	Cancer development is accelerated by cell migration and the stimulation of the epithelial-mesenchymal transition.	11q22.3	
		Gelatinases		
Gelatinase A	MMP-2	the growth of axons, cell motility, mesenchymal cell differentiation into an inflammatory phenotype, an increase in the bioavailability of transforming growth factor-, migration of epithelial cells, anti- inflammatory, neuronal apoptosis resulting in neurodegeneration; in the development of cancer, cleavage of insulin-like growth factor-1 binding proteins, proliferation	16q13	Elastin, fibronectin, gelatin, and collagen (4 to 6, 10).

Gelatinase B	MMP-9	A few effects include collagenous affinity, pro-inflammatory and anti-inflammatory behavior, tumor cell resistance, decreased Interleukin-2 reaction, hypertrophic chondrocyte cell death and the incorporation of new functional osteoblast units, and stimulation of Transforming growth factor-in tumor formation.	20q11.2-q13.1	Gelatin, elastin, fibrillin, collagens 4, 5, 7, 10, and 15, and osteonectin
Stromelysins				
Stromelysin-1	MMP-3	Cell migration, programmed cell death of an epithelial cell, epithelial-mesenchymal transition, epithelial bubble formation, angiostatin-like element production, enhancing affinity for collagen, the release of bFGF, increase in insulin-like growth	11q23	Gelatin, fibronectin, laminin and aggregate
		factor-1 bioavailability, cell proliferation, pro-inflammatory and anti-inflammatory activity, increase in Transforming Growth Factor- bioavailability, a disorder in cells aggregation, and cellular invasiveness are all factors that contribute to the progression of cancer		

Stromelysin-2	MMP-10	Degradation of collagen-4, 10,7, and perlecan in the advancement of cancer; production of tumstatin, endostatin,angiostatin, and endorepellin	11q22.3-q23	Gelatin, casein, aggregan, elastin, collagens (3 to 5), MMP-1 and MMP-8
Stromelysin-3	MMP-11	In the process of cancer, a 1-proteinase inhibitor is released, which lowers the cancer cell's sensitivity to natural killer cells.	22q11.2	Gelatin, aggregan, laminin, and fibronectin
Matrilysin	MMP-7	Cell differentiation enhanced affinity for collagen, enhanced bioavailability of insulin- like growth factor-1 and Transforming Growth Factor-beta, inappropriate cell aggregation and invasiveness, cell death brought on by stimulation of the Fas receptor, pro-inflammatory initiation of osteoclast activity, vasculature constriction, and cell proliferation are some of the other factors that affect tissue.	11q21-q22	Gelatin, fibronectin, laminin, collagen 6 to 10), aggregan, and pro-MMP-9
Metalloelastase	MMP-12		11q22.2-q22.3	Collagen 1, 4, fibronectin, laminin, vitronectin, proteoglycan, gelatin,elastin, and others

Matrily in -2	MMP-26		11p15	Collagen 4, pro-MMP-9, and gelatin
***Membrane-type MMPs* (*MT-MMP*)**				
MT-MMP-1	MMP-14	Epithelial cell motility, decreased adhesion, diminution cell flattening, anti-inflammatory properties, and trailers of the embryos to the uterine epithelium	14q11-q12	Gelatin, fibronectin, laminin aggrecan, tenascin and collagen (1,2, and 3)
MT-MMP-2	MMP-15	Reduced cell flattening and adhesion	15q13-q21	Laminin, aggrecan, perlecan, fibronectin
MT-MMP-3	MMP-16	Reduced cell flattening and adhesion	8q21	Collagen 3, gelatin, casein
MT-MMP-4	MMP-17		12q24.3	Fibrinogen and tumor necrosis factor precursor
MT-MMP-5	MMP-24		20q11.2	Proteoglycans

Tissue inhibitor of Metalloproteinase (TIMP)

MMP activity is regulated by the endogenous inhibitor MMP, the tissue inhibitor of metalloproteinase (TIMPs)

IV.Tissue inhibitor of Metalloproteinase

MMPs	**Inhibitor TIMP**
MMP-1, MMP-3, MMP-7	TIMP-1
MMP-2	TIMP-2
MMP-2	TIMP-3
MT-1 MMP AND MMP-2	TIMP-4

TIMP-1 and TIMP-2 are two of these TIMPs, become more significant in cardiovascular disorders linked to obesity. Because the balance between MMPs and TIMPs is a crucial factor in determining the integrity and function of the ECM, changes in MMPs/TIMPs-mediated proteolysis may lead to various clinical disorders. Changes in TIMPs levels are crucial in pathological circumstances because they directly impact MMP activity. [7]

1.7 OXIDATIVE STRESS AND HTN:

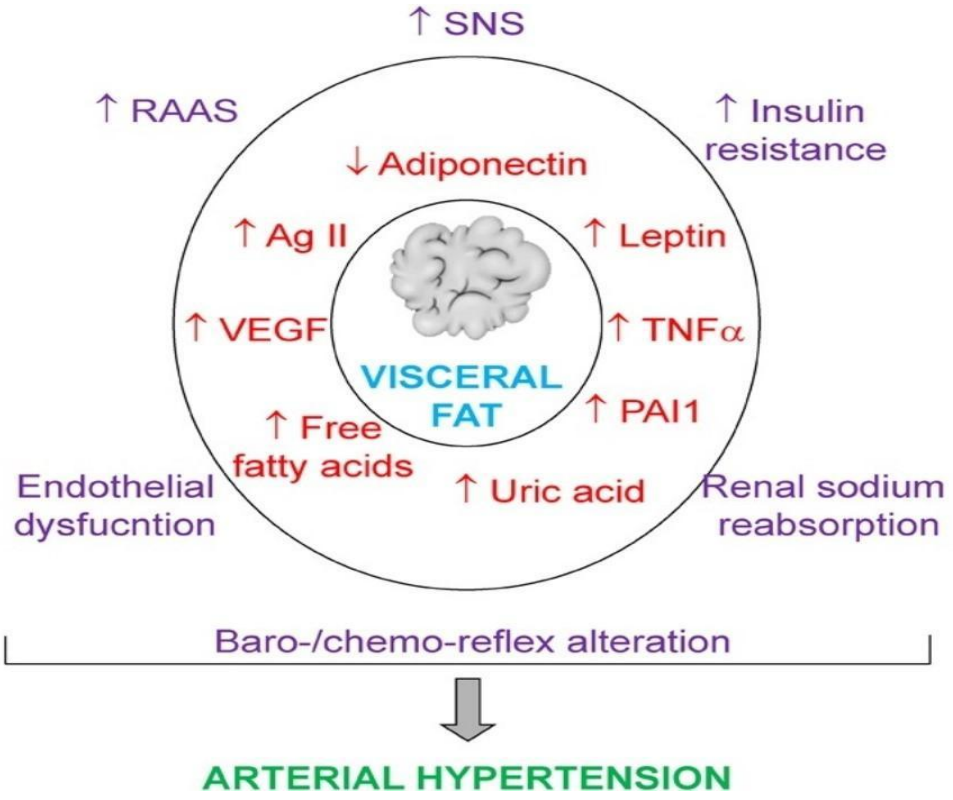
The development of HTN depends on vascular oxidative stress. It also foresees an increase in CVD. [6] Oxidative stress results from an imbalance between the body's ability to produce reactive oxygen species (ROS) and protect itself from them using antioxidants. ROS are oxygen-containing, chemically reactive substances that have yet to undergo complete digestion. Examples of ROS include singlet oxygen, hydrogen peroxide (H_2O_2), hydroxyl radicals (OH), and superoxide radicals (O^2-). Numerous enzymes in various cell compartments

produce lots of ROS. However, most ROS are generated in the mitochondrial matrix during the ATP-producing oxidative phosphorylation event.[54] The cell membrane and macromolecules such as proteins, DNA, and RNA may be harmed if the level of ROS is not regulated within a healthy range. [55]By using substances referred to as antioxidants, our body can detoxify or fight the harmful effects of ROS and maintain them at a healthy level. Our bodies either create antioxidants on their own (endogenous antioxidants) or consume them from the outside world (exogenous antioxidants). SOD, catalase, glutathione peroxidase, and glutathione reductase are some enzymes acting as antioxidants. Glutathione has been reduced. Minerals include, among others, selenium, manganese, copper, and zinc. Examples of vitamins include vitamins A, C, and E. Reactive oxygen species impact cell proliferation and inflammatory responses. Through reduction-oxidation-dependent signalling pathways, which affect cardiovascular structure and function.[56] The endothelium is destroyed by increased oxidative stress in the arteries, which inhibits Endothelium Nitric oxide synthase (eNOS) pathways, decreases nitric oxide synthesis, and interferes with endothelium- dependent vasodilation, all of which contribute to the high vascular tone and, ultimately, hypertension. Additionally, oxidative stress results in smooth muscle cell proliferation and hypertrophy, which thickens the vascular medium and reduces the artery lumen. These findings imply that oxidative stress may substantially impact the onset of hypertension. [13]

Many studies have been done in this area that proves oxidative stress is responsible for HTN, but the relationship between MMP-9, TIMP-1, and oxidative stress remains unclear. Due to the paucity of work in this part of Karnataka this study is undertaken. So this study's objective is to examine the blood serum levels of MMP-9 and TIMP-1 and their ratios in numerous study populations, including obese and non-obese individuals with HTN and healthy individuals with or without obesity. Additionally, correlations between oxidative stress, several anthropometric measurements, blood pressure, and MMP-9 and TIMP-1 levels.

Review of literature

HTN is a significant comorbidity that influences the development of cardiovascular disease, renal failure, myocardial infarction, and stroke. It was one of the issue that received the most investigation throughout the previous century. The most prevalent kind of hypertension is idiopathic or essential hypertension. It is the most common kind of arterial hypertension in adults. Globally, hypertension is turning into a significant issue that has an impact on the adult population's health and welfare.[14]The intermediate phenotype of primary hypertension is due to altered body composition, including visceral fat deposition, faster biological maturation, metabolic problems indicative of metabolic syndrome, and enhanced adrenergic drive.[15]The chance of dying from a stroke, heart attack, or other vascular illness doubles with every 20 mmHg increase in systolic blood pressure over normal blood pressure.[14] Obesity and hypertension (HTN) are linked to metabolic syndrome and are pretty standard in people with CVD. It has the role in the development of heart failure (HF) and various pathophysiologic pathways underlie obesity-related high blood pressure. [16]



SNS- Sympathetic nervous sysytem

RASS-renin-angiotensin system

VEGF-Vascular endothelial growth factor

Ag II-Angiotensin II

TNF-α- Tumor necrosis factor

A recently published data based on comparative research on body fat in a group with and without hypertension. Three groups were tested for height and weight and discovered substantial changes between normal and high blood pressure populations. The average BMI, WC, WHtR, and the gap between body fat % and visceral fat area, were calculated. They concluded that there were substantial variations between hypertension and normotensive persons in terms of BMI, WC, WHtR, body fat percentage, and visceral fat area (Rang Wang et al., 2021).[17] Using data from the Survey conducted in 2015–2016 National Family Health carried out across from January 2015 to December 2016: India, Vishal Vennu et al. reported their findings in 2019. According to age group, sex, and type of habitation, calculations were produced for the prevalence and prevalence rate per 100,000 persons at the national level and for each state and union territory.[18]

There were 14.6%, 3.4%, and 5.2% of Indians who were overweight, obese, and hypertensive, respectively. At the national level, those between 35 to 49 years old and particularly women of metropolitan areas had the highest chances of these conditions/diseases. In India, the nationwide prevalence of overweight, obesity, and hypertension was 1 out of every 7, 29, and 14 people, respectively, during 2015 and 2016. [18]

MMP-9 AND HYPERTENSION

Through the breakdown and modification of ECM components, MMPs are engaged in pathophysiological problems of obesity or metabolic syndrome. TIMP, an endogenous inhibitor of MMPs, controls their activity. TIMPs levels directly influence MMPs activity, and alterations in TIMPs levels are significant in pathological circumstances. Obesity and HTN largely depend on the harmony between MMP and their TIMP levels. An imbalance between the MMP and their natural inhibitors has been discussed in several circumstances linked to HTN-related complications. Although this connection is still unclear, several investigations have demonstrated that MMP and TIMP concentrations relate to anthropometric measurements and endothelial functions in fatness. According to research by Karim Chahed et al., published in 2021. In obese, overweight and non-obese individuals without metabolic syndrome, investigators assessed the serum concentration of MMP-1, MMP-2, and its ratios. It also looked at the relationship between MMPs and TIMPs concentrations and several anthropometric measures, blood pressure, and endothelial function. They concluded that MMP-1, MMP-3, and MMP-9 concentrations are connected to BMI, WC, hypertension, and endothelial-dependent response, among other obesity-associated characteristics. [19] In another study, Alejandro F. Proda et al, 2021 determined the relationship between matrix metalloproteinase activity and high blood pressure. They discovered that patients with high blood pressure have higher MMPs than healthy individuals and they observed Individuals with hypertension have higher MMP-1, MMP-2, MMP-3, and MMP-9 than normal. [20]

Using age-matched normotensive and hypertensive children as reference. Joanna B. Trojanek et al. conducted a research project in 2020 to examine MMP/TIMP

gene expression in peripheral blood leukocytes of hypertension children. They discovered that the word MMP-2 was higher in hypertension children than in normal controls, regardless of age, gender, or body composition. TIMP-2 expression was linked with the carotid wall cross-sectional area. In contrast, MMP-14 expression was higher in individuals with left ventricular hypertrophy than in patients with normal left ventricular mass. They concluded that, compared to normotensives, children with hypertension had substantial changes in MMP/TIMP expression patterns. Alterations in MMP/TIMP expression have also been linked to metabolic problems and hypertensive target organ damage. [21] Changes in the activity of circulating MMP-2 and MMP-9 in patients with heart failure about gender, hypertension, and treatment: cross-sectional research was carried out by E Giannakos in 2016. They concluded that pro-MMP-2 activity might be inhibited by antihypertensive therapy. The suppressive impact of antihypertensive medication on pro-MMP-2 activity is diminished When heart failure and hypertension coincide. Their findings also point to the possible influence of cardio-preventive factors on the activity of pro-MMP-2 in females [22]

In the study of MMP-2 and MMP-9 Function in the treatment of Resistant Hypertension Patients', they observed that neither MMP-2 nor MMP-9 impacts BP regulation in treatment-resistant HTN individuals, which was followed by X. Lacerda et al. In 2015.[23] In 2009, Vanessa A. et al. compared the pro-MMP-2, pro-MMP-9, total MMP-9 and MMP-8 levels in the blood and their endogenous inhibitors- TIMP-1 and 2 along with MMP-8/TIMP-1, MMP-9/TIMP-1, and MMP-2/TIMP-2 ratios, were also compared in normotensive obese children and adolescents. They concluded that there is evidence of increased net MMP-8 activity in childhood obesity but not MMP-9 or MMP-2. The elevated MMP-8 levels in obese kids point to a potential pathophysiological mechanism responsible for the high risk of coronary heart disease linked to juvenile obesity. [24]

In 2008, Ibrahim Koral Onal et al. observed the effects of antihypertensive therapy on the levels of the blood enzymes MMP-9 and TIMP-1 in hypertension and normotensive persons. The hypertensive group's serum MMP-9 and TIMP-1 levels were evaluated before and after a 3-month antihypertensive therapy. They concluded that elevated MMP-9 activity in hypertensive patients could lead to increased elastin

degradation relative to collagen and non-elasticity. While decreased TIMP-1 activity could result in an accumulation of poorly cross-linked, immature, and unstable fibril degradation products, leading to misdirected collagen deposition. [25] Systolic hypertension and arterial stiffness are correlated with MMP-9, MMP-2, and serum elastase activity, respectively.according to 2005 research by Yasmin et al. The Serum elastase activity (SEA), lipids, C-reactive protein, MMP-9, MMP-2, and TIMP-2 were assessed. They concluded that MMP-9 levels and SEA in Isolated systolic HTN and younger physically healthy persons are connected to Aortic stiffness. This shows that MMP-9 may be involved in the formation of the process of arterial stiffening. [26]

OXIDATIVE STRESS AND HYPERTENSION

It has been recently suggested that one of the fundamental causes of hypertension is oxidative stress. Oxidative stress is brought on by a rise in the formation of reactive oxygen species, which include superoxide radicals (O2), hydrogen peroxide (H2O2), hydroxyl radicals (•OH), and singlet oxygen.[13] Vascular matrix metalloproteinase (MMP) activity will rise in response to elevated oxidative stress and decreased nitric oxide (NO) bioavailability. It has been investigated that NO, MMP, and oxidative stress interact to cause vascular changes associated with hypertension. Although this pathophysiological process cannot entirely account for the changes caused by hypertension. Substantial evidence supports that these pathways are crucial in raising vascular MMP expression and activity,Strong evidence suggests that these pathways play a crucial role in increasing the expression and activity of vascular MMP, which causes the abnormal degradation of extracellular matrix components, receptors, peptides, and intracellular proteins that regulate vascular shape and function. Cell expansion or migration, related to the deposition of extracellular matrix components, is facilitated by stimulating vascular smooth muscle cells (VSMC) to switch from contractile to synthetic phenotypes. Vascular MMP activity is unbalanced, which promotes vasoconstriction and prevents vasodilation by activating VSMC.[27]

Research on the role of oxidative stress in the emergence of obesity and obesity-related metabolic illnesses was undertaken in 2021 by Emina Colak et al. They

discovered that oxidative stress, which stimulates the deposition of fatty tissue, including pre-adipocyte proliferation, adipocyte differentiation, and growth, plays a causal role in the development of obesity. Exercise-induced weight reduction might enhance redox by modifying oxidative stress and antioxidant promoters, which lessen endothelial dysfunction and inflammation. [28]

To investigate the link between anthropometric and biochemical factors and oxidative stress in young persons with healthy metabolisms, poor metabolisms, and average weight., Grzegorz K. Jakubiak et al. undertook research in 2021. TAC(Total antioxidant capacity) , TOS(total oxidative status), OSI(oxidative stress index) , serum concentrations of MDA, ceruloplasmin, thiol groups and LPH (lipid hydro peroxides), the concentration of LPS (lipofuscin) in erythrocytes, and superoxide dismutase activity were among the parameters of oxidative stress that were measured. They concluded that there were substantial differences between young volunteers with average body weight and no relevant health information and MUO (metabolically unhealthy obese) persons in the chosen indices of oxidative stress. And in contrast to those with normal weight and no metabolic abnormalities, their findings show that metabolically unwell obese patients have more significant oxidative stress measures. [29]

Julia Krzemińska et al., in 2020, conducted a study on Arterial Hypertension (AH) Inflammation and oxidative stress. They focused on how oxidative stress and inflammation, influenced by various variables, including nutrition, supplements, and medication, relate to AH. They concluded that a single dietary addition might lower blood pressure, but it is uncertain how particular foods' antioxidant/anti-inflammatory qualities affect how hypertensive they are. Furthermore, AH pharmacotherapy reduces oxidative stress, which may aid in the prevention of organ damage. [30] Lucas C. Pinheiro et al. in 2020 saw the completion of a study on the Causes and Consequences of Oxidative Stress in Hypertension. They concluded that endothelial dysfunction, vascular remodelling, and tissue damage were the significant impacts of oxidative stress in hypertension, followed by the primary antioxidant defences in the vasculature. [31]

According to the most recent European Society of Cardiology/European Society of Hypertension (ESC/ESH) recommendations, research by Hala Shokr et al. in 2020 looked at retinal vascular function and how it relates to the body's ability to fight oxidative stress in normal vs. early hypertension persons. Each participant's blood pressure, lipid panel, and oxidized and reduced glutathione levels were assessed. Their findings show that microvascular changes may still be detected in blood pressure readings in normal ranges. These changes correspond to variations in the degree of oxidative stress. [32] In this regard, To assess the serum NO and MDA levels in the hypertensive population, Dr. Yale BM et al. did research in 2019. They consider sex- and age-matched hypertensive and normotensive people. Among whom anthropomorphic parameters, NO, and MDA were measured. And concluded that the relationship between impaired NO, bioactivity-increased MDA, and hyper-cholesterol needed to be clearly understood. Therefore, more research is required to understand how NO bioactivity affects the concentrations of MAD in high blood pressure and how to control this condition. [33]

In 2008, H.D. Khanna et al,. done research on oxidative stress in hypertensive people. They evaluated any potential confounding effects from three months of antihypertensive medication. They discovered that serum MDA levels were considerably higher than the control groups. The superoxide dismutase, glutathione, vitamin E and C levels are decreased in hypertensive. They concluded that antihypertensive medications would lower blood pressure and lessen oxidative stress. It is proved that oxidative stress is not the cause of hypertension but rather one of its effects. [34]

HYPOTHESIS

There may be a correlation between obesity and hypertension versus Matrix metalloproteinase-9 and Tissue inhibiting matrix metalloproteinase-1.

AIMS & OBJECTIVES OF THE STUDY:

AIM :

To evaluate serum MMP-9 and TIMP-1 levels in obese and non-obese hypertension and non- hypertension individuals.

OBJECTIVES :

1. Evaluation of the influence of MMP-9 & TIMP-1 in obese and non-obese Hypertensive and Non-Hypertensive individuals.
2. Study the correlation between Anthropometric parameters ,MMP-9 and TIMP-1in obese and non-obese Hypertensive and Non-Hypertensive individuals.
3. Study the correlation between Oxidative stress with MMP-9 & TIMP-1in obese and non-obese Hypertensive and Non-Hypertensive individuals.

MATERIALS AND METHODS

The present study was carried out in the department of biochemistry and medicine, BLDE Deemed to be University University Sri B.M.Patil medical college and research center, Vijayapura.

1. SAMPLE SIZE

With the Anticipated correlation between MMP-9 and Cardiovascular complications (CVC) in people with obesity

r= -0.47 [19] at 95%confidence level and 90 powers in the study, the sample size worked out is the formula used is

$$N=\left[\left(\frac{Z_\alpha+Z_\beta}{C}\right)\right]^2+3$$

The standard normal deviate for α = Zα = 1.9600

The standard normal deviate for β = Zβ = 1.2816

C = 0.5 * ln [(1+r)/ (1-r)] = 0.5101

N=53+53=106

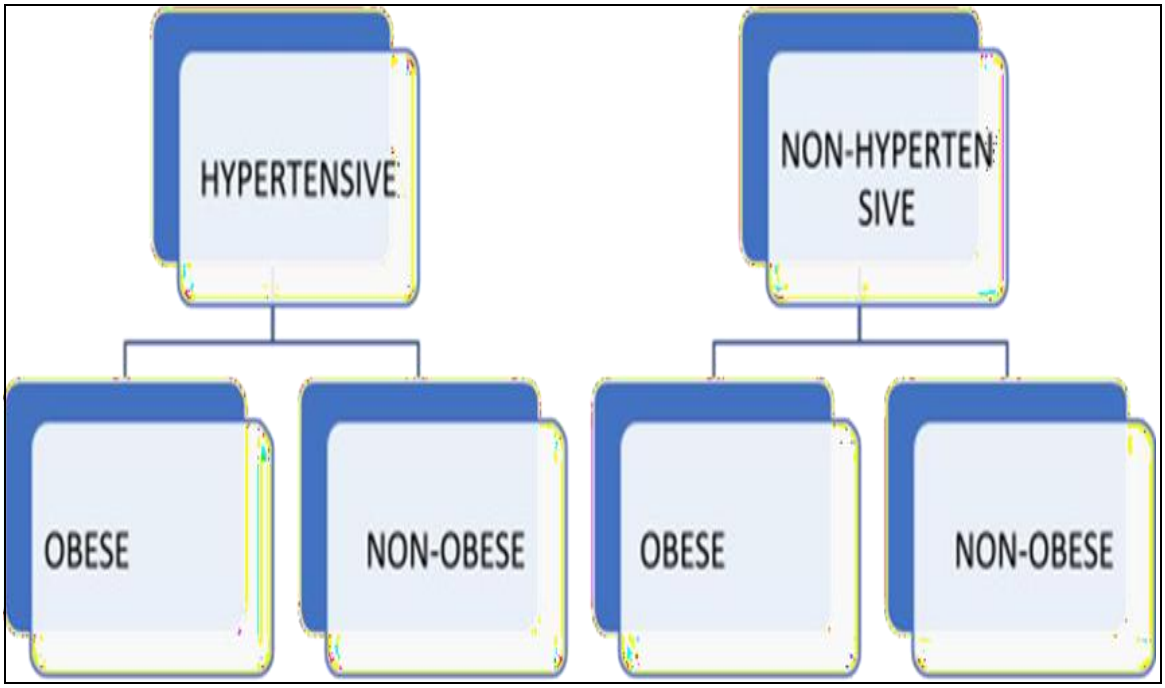
53 hypertensive patients were compared with the same number of normotensive age and sex matched subjects.

Hence 106 participants were unrolled in the study.

2. STUDY participants and distribution:

- Group-1: Hypertensive/ study group: The study group consists of 53 subjects including both obese and non-obese males and females of age 51±14 years.

● Group II: Non-hypertensive/Control group: The control group consists of 53 subjects including both obese and non-obese males and females of age 51±14 years.



3. INCLUSION CRITERIA:-

●Study group: The male and females aged 51±14 years with systolic blood pressure (SBP) ≥140 mmHg and Diastolic blood pressure (DBP)≥ 90 mmHg

●Control group: The male and females aged 51±14 with SBP ≤ 120mmHg and DBP ≤80mm Hg will be included.

4. EXCLUSION CRITERIA: -

- Hypertensive Patients with Renal failure
- Stroke
- Diabetes
- Liver diseases
- Gout
- Patients who disagreed with donating blood samples
- Thyroid disease
- Smokers
- Alcoholic
- Pregnant women
- Women on menstrual cycle.

5. ETHICS:

The Shri B M Patil Medical College, Hospital, and Research Center's institutional ethical committee approved the study. The participants' signed agreement was received after explaining the study project's goal and methodology.[page no -98]

6. MEASUREMENT OF BLOOD PRESSURE

Using a mercury sphygmomanometer, blood pressure was recorded three times at intervals of two minutes while the subject was seated. Took the three recordings' averages into account.

7. METHODS OF SAMPLE COLLECTION

1) To conduct the study, a blood sample from hypertensive patients who were referred to the biochemistry department at Shri B M Patil Medical College in Vijayapura would be collected.

2) The participant was given a consent form to get the necessary information.

3) The patient's consent form was filled out and given instructions to the subject before obtaining the sample.

4) Offered consent forms both in Kannada and English.

5) A venous puncture was used to obtain a sample of 5 ml of fasting venous blood under aseptic conditions.

6) The blood was centrifuged for 10 minutes at 3000 rpm to separate the serum, which was then be collected in a polythene tube and kept at -80°C until the analysis is performed. An empty polythene tube is used to collect serum.

Transport of sample: all the samples will be transported immediately to the laboratory and processed within 24 hours in order to secure accurate and reproducible results.

8. BIOCHEMICAL PARAMETERS

8.1 ESTIMATION OF MMP-9

AIM: To estimate the serum MMP-9 levels

Test principle

The micro-ELISA plate has been pre-coated with an antibody specific to human MMP-9, according to the sandwich-ELISA concept. Fill the micro-ELISA plate wells with samples (or standards) and the particular antibody. After that,combine a biotinylated detection antibody with the avidin-horseradish peroxidase HRP mixture. Washing involves the removal of unbound components. The substrate solution is poured into each well. Only the wells containing Human MMP-9 biotinylated detection antibody and HRP conjugate will be coloured blue. The colour turns yellow when an enzyme-substrate reaction is stopped by the addition of a stop solution. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm. Human MMP-9 concentration and optical density have an antagonistic relationship. One can estimate the amount of human MMP-9 present in the samples by comparing the optical density of the samples to the standard curve.

Sample collection:

Serum: Centrifuge samples for 20 minutes at 1000 rpm after letting blood clot at room temperature. To conduct the test, collect the clear supernatant.

Reagent preparation

1. Before using, bring all reagents to room temperature 28℃.

2. Wash Buffer: To make 750mL of Wash Buffer, combine 30mL of Concentrated Wash Buffer with 720mL of deionized or distilled water.

3. Usual and practical response Centrifuge the standard for one minute at 10,000 rpm. Invert it gently several times after adding 1.0 mL of Reference Standard & Sample

Diluents and letting it stand for 10 minutes. Then, as necessary, produce further dilutions. The following is the suggested dilution gradient: 10,5, 2.5, 1.25,0.63, 0.32, 0.16 ,0 ng/mL.

Method of dilution: Take 7 EP tubes and fill each one with 500 microliters of the reference standard and sample diluent. 500 microliter of the 10ng/mL working solution should be pipetted into the first tube before mixing to create the 5ng/mL working solution. Following this step, pipette 500 microliter of the solution from the first tube and transfer it into the second tube.

1. The effective treatment for biotinylated detection antibody: Concentrated Biotinylated Detection antibodyshould be centrifuged at 800 g for one minute before diluting from 100 to 1 working solution with Biotinylated Detection antibody diluent.

5. To create the HRP Conjugate working solution, centrifuge the concentrated HRP conjugate at 800 g for one minute. Then, dilute the concentrated HRP conjugate from 100 to 1 by seven times.

Assay procedure

1. Place 100 µL of the appropriate standard, blank, and sample into the wells, then incubate for 90 min at 37 °C.

2. Remove only the liquid from each well and decant it. Immediately add 100 µL of the Biotinylated detection antibody working solution to each well. At 37 °C, incubate for one hour.

3. After adding 350 µL of wash buffer to each well, decant the solution from each well. Aspirate or decant the solution from each well after soaking for 1-2 minutes.

4. Add 100 micro litter of the HRP Conjugate working solution into each well. Refresh the sealer on the plate. Incubate for 30 minutes at 37 °C.

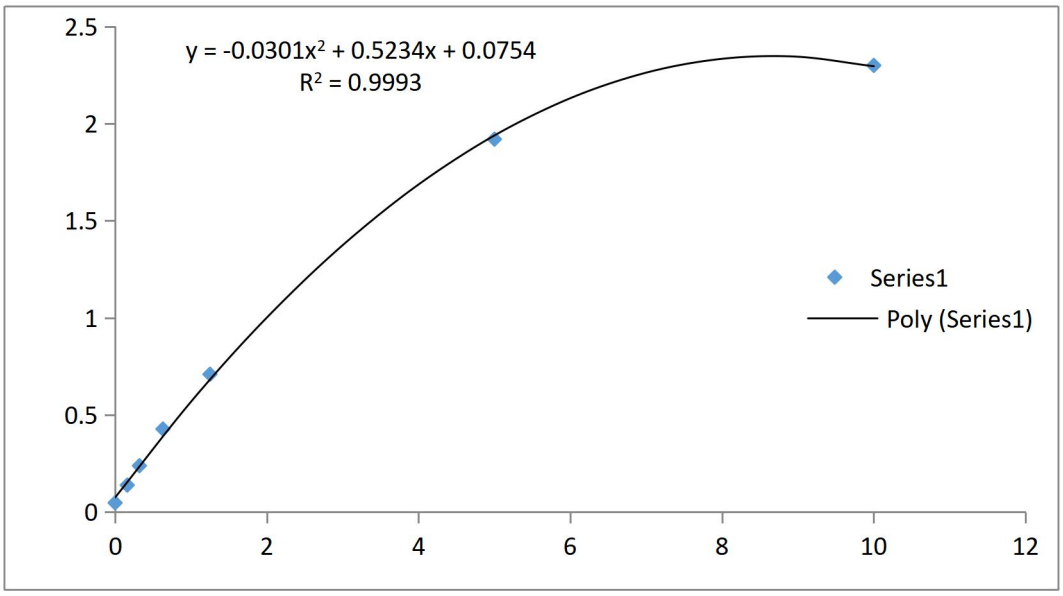
5. Wash for five times

6. Fill each well with 90 µL of the substrate reagent. Apply fresh sealer to the plate. At 37°C, incubate for around 15 minutes. Keep the plate out of the light.

7. Fill each well with 50 µL of Stop Solution.

8. Measure the optical density

Calculation of results: Average each standard's duplicate measurements. With a standard concentration on the x-axis and OD values on the y-axis, draw a four-parameter logistic curve on the log-log graph paper. The dilution factor is multiplied by the computed concentration to determine the actual concentration.



9.2.ESTIMATION OF TIMP-1

AIM: to estimate the serum TIMP-1 levels

Principle:

The micro-ELISA plate has been pre-coated with an antibody specific to human TIMP-1, according to the sandwich-ELISA concept. Fill the micro-ELISA plate wells with samples (or standards) and the particular antibody. After that,combine a biotinylated detection antibody with the avidin-horseradish peroxidase HRP mixture. Washing involves the removal of unbound components. The substrate solution is poured into each well. Only the wells containing Human TIMP-1 biotinylated detection antibody and HRP conjugate will be coloured blue. The colour turns yellow when an enzyme-substrate reaction is stopped by the addition of a stop solution. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm. Human TIMP-1 concentration and optical density have an antagonistic relationship. One can estimate the amount of human TIMP-1 present in the samples by comparing the optical density of the samples to the standard curve.

Sample collection

Serum: Centrifuge samples for 20 minutes at 1000 g after letting blood clot at room temperature. To conduct the test, collect the clear supernatant.

Reagent preparation

1. Before using, bring all reagents to room temperature 28℃.

2. Wash Buffer: To make 750mL of Wash Buffer, combine 30mL of Concentrated Wash Buffer with 720mL of deionized or distilled water.

3. Usual and practical response Centrifuge the standard for one minute at 10,000 g. Invert it gently several times after adding 1.0 mL of Reference Standard & Sample diluent and letting it stand for 10 minutes. Then, as necessary, produce further dilutions. The following is the suggested dilution gradient: 10, 5, 2.5, 1.25, 0.63, 0.32, 0.16, 0 n g/mL.

Method of dilution: Take 7 EP tubes and fill each one with 500 micro liters of the reference standard and sample diluent. 500 micro liter of the 10ng/mL working solution should be pipetted into the first tube before mixing to create the 5ng/mL working solution. Following this step, pipette 500 micro litter of the solution from the first tube and transfer it into the second tube.

4. The effective treatment for biotinylated detection antibody: Concentrated Biotinylated Detection antibody should be centrifuged at 800 g for one minute before diluting from 100 to 1 working solution with Biotinylated Detection antibody diluent.

5. To create the HRP Conjugate working solution, centrifuge the concentrated HRP conjugate at 800 g for one minute. Then, diluent the concentrated HRP conjugate from 100 to 1 by seven times.

Assay procedure

1. Place 100 µL of the appropriate standard, blank, and sample into the wells, then incubate for 90 min at 37 °C.

2. Remove only the liquid from each well and decant it. Immediately add 100 µL of the Biotinylated detection antibody working solution to each well. At 37 °C, incubate for one hour.

3. After adding 350 µL of wash buffer to each well, decant the solution from each well. Aspirate or decant the solution from each well after soaking for 1-2 minutes.

4. Add 100 micro litter of the HRP Conjugate working solution into each well.

Refresh the sealer on the plate. Incubate for 30 minutes at 37 °C.

5. Wash for five times

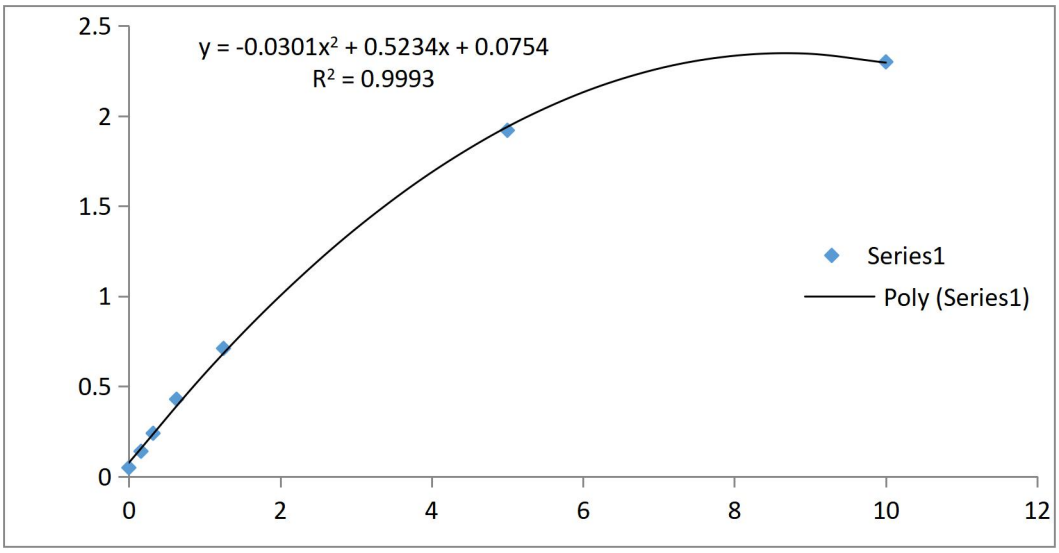
6. Fill each well with 90 µL of the substrate reagent. Apply fresh sealer to the plate. At 37°C, incubate for around 15 minutes. Keep the plate out of the light.

7. Fill each well with 50 µL of Stop Solution.

8. Measure the optical density at..... nm

Calculation of results

Average each standard's duplicate measurements. With a standard concentration on the x-axis and OD values on the y-axis, draw a four-parameter logistic curve on the log-log graph paper. The dilution factor is multiplied by the computed concentration to determine the actual concentration.



9.3. Estimation of Serum and Tissue Malondialdehyde (MDA) [13]

By the method of Buege and Aust (Buege and Aust, 1978)

• Introduction

Malondialdehyde (MDA) is a marker of oxidative stress in biological systems. It is a by product of the lipid peroxidation process. It is created when a free radical chain reaction breaks down polyunsaturated fatty acids (PUFA). It is one of the several reactive electrophile species that cause oxidative stress and is a reactive aldehyde.

• Principle

MDA, produced when PUFA breaks down, is an easy method for determining how much lipid peroxidation has occurred. It interacts with TBA to produce a pink hue that may be detected at 535 nm.

Sample: serum

Chemicals required:

1. Trichloro acetic acid (TCA)
2. 2-Thiobarbituric acid (TBA)
3. Hydrochloric acid (HCl)
4. Malonaldehyde bis (dimethyl acetal)

• Preparation:

1. 0.25 N HCl: TCA-TBA-HCl Reagent Using pure water, 2.21 ml of concentrated HCl was diluted to 100 ml (DW). In 100 ml of 0.25 N HCl, 15% TCA and 0.375% TBA were dissolved. Warming up the reaction mixture let the ingredients dissolve, and it was then kept at 4°c
2. MDA stock standard -164 µg/ml:16.4 µl of standard MDA solution was taken and made up to 100 ml with D/W.
3. MDA working standard (working- 1.64 µg/ml):100 µl of the stock was made up to 10 ml with D/W.

Table no.VI

SNo	Vol. of MDA (ml)	Vol. of D.H2O (ml)	Conc. Of MDA (µM/L)	TBA-TCA-HCl Reagent (mL)	Keep in boiling water bath for 15 minutes
B	0.0	1	0.0	1	
1	0.2	0.8	2.0	1	
2	0.4	0.6	4.0	1	
3	0.6	0.4	6.0	1	
4	0.8	0.2	8.0	1	
5	1	-	10.0	1	

• **The procedure for standardization: Standardization (Range 2-10 µM/L)**

The standardization was carried out by referring to the table and all the reagents were added according to the following table. Read O.D absorbance at 535 nm. The optical densities were plotted against the concentration on a graph.

Estimation of MDA in the sample:

• Sample preparation:

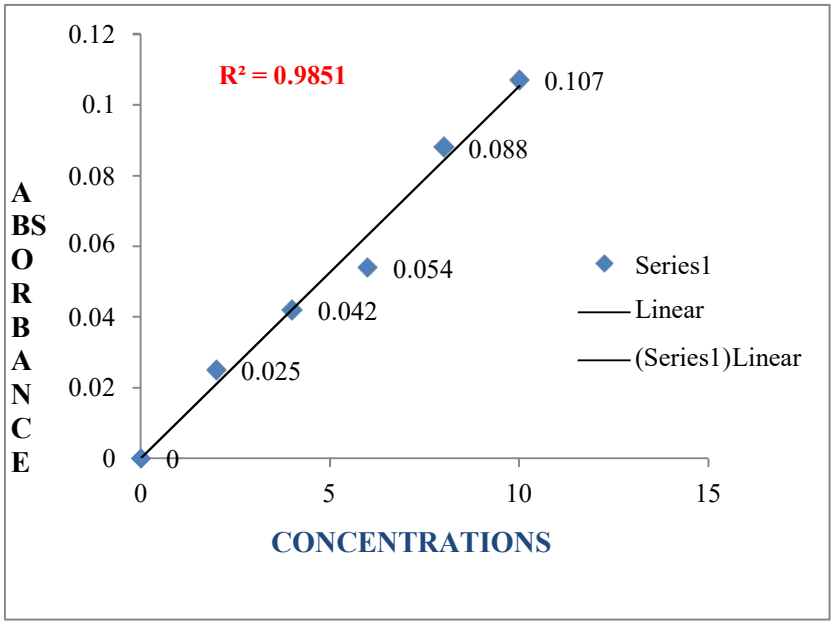
Serum: 100 µl + 500µl with distilled water

• Procedure

1. 1µl of TCA-TBA-HCl reagent was applied to the diluted sample.
2. For 15 minutes, the samples were maintained in a bath of boiling water.
3. The reaction mixture was centrifuged after cooling.
4. The optical densities of the pink colour generated in the supernatant were measured at 535 nm by a UV-visible spectrophotometer (Shimadzu, Model: UV1800).
5. By comparing the acquired absorbance to the norm, the amount of MDA in the sample was calculated. The optical density of the pink colour that resulted was closely correlated with the amount of MDA present in the sample.

• **Calculations**

The optical densities of the test samples were directly proportional to the concentration of MDA in the sample and calculated by plotting against the standard graph and multiplying by the respective dilution factors. The final concentration was expressed as µM/L.



9.4. Estimation of Serum, and Tissue Nitric Oxide concentration[13]

By Griess Reaction (Moshage Han et al., 1995; Cortas and Wakid, 1990; Green et al., 1982)

• **Principle**

Total NO2/NO3 is usually used to measure the amount of NO because it is unstable and can be transformed into nitrite and nitrate. Nitrite was formed from nitrate. Vanadium chloride (VCl3) is used with the Somogyi reagent (NaOH & ZnSO4), which deproteinized the serum sample. To determine how much nitrite was produced, sulphanilamide was diazotized and linked to naphthalene diamine. After deproteinization

and coupling to N-naphthyl ethylenediamine, nitrate—the stable by-product of nitric oxide—was reduced to nitrite by the cadmium reduction method. In a spectrophotometer, the coloured complex was measured at 540 nm.

Reagents

1. Granules of cadmium: 2.5–3 gm in 0.1 M/L H_2SO_4

2. Glycine-NaOH buffer (pH-9.7): 200µL of distilled water was used to dissolve 7.5 grams of glycine. The pH was then brought down to 9.7 by adding 2M NaOH, and 500 ml of distilled water was diluted.

3. Sulphanilamide: 2.5 grams of sulphanilamide were dissolved in 250 µL of heated 3M/L HCl and then cooled.

4. N-Naphthyl ethylenediamine: 50 mg Distilled water was used to dissolve N-naphthyl ethylenediamine, and the volume was then made to 250ml.

5. NaNO2, or standard sodium nitrite solution:

a) 690 mg of sodium nitrite was dissolved in 100 ml of a 10 mmol/L of sodium borate to create the stock standard (0.1mol/L).

b) Working standard (10 mol/L): Sodium nitrite ($NaNO_2$) stock solution (10 µl) was diluted to a final volume of 100 ml with sodium borate solution (10 mol/L).

6. A 75 mmol/L solution of $ZnSO_4$

7. A 55 mmol/L solution of NaOH

8. A 0.1 mol/L solution of H_2SO_4

9. 125 mg of $CuSO_4$ was dissolved in 100 ml of the glycine-NaOH buffer to create a $CuSO_4$ solution (5 mmol/L).

• **Procedure**

I. **Deproteinization:**

- Draw 0.5 ml of serum into a centrifuge tube that is clean and dry.
- Mix in 2.0 ml of the 75 mmol/L $ZnSO_4$ solutions.
- Centrifuge for 10 minutes after adding 2.5 ml of the 55 mmol/L NaOH reagent and thoroughly mixing.
- The supernatant is handled as a filter free of protein.

II. Activation of cadmium granules:

- At the time of the analysis, cadmium granules that had been previously kept in 0.1mol/L H_2SO_4 solution were three times rinsed with distilled water.
- After that, the granules were stirred for a few minutes in 5 mmol/L $CuSO_4$ solutions.
- The glycine-NaOH buffer was used to drain and wash the copper-coated granules.
- After activation, these granules were used within 10 minutes.
- After use, the granules were rinsed with distilled water and placed in a 0.1 mmol/L H_2SO_4. Each time, the exact identical granule activation process was used.

III. Nitrite Assay:

1. Three Erlenmeyer flasks were used, each with the labels Blank, Test, and Standard
2. Each Erlenmeyer flask received 1 ml of glycine-NaOH buffer. 1 ml of deionized water, 1 ml of the deproteinized sample, and 1 ml of the working standard were added to the flasks marked B (Blank), T (Test), and S (Standard), respectively.
3. Each flask was filled with 2.5 - 3 grams of newly activated cadmium granules using a spatula.
4. The granules were mixed in all of the flasks' contents.
5. The mixture in each of the three flasks was diluted to 4 ml with distilled water after 90 minutes.

6. From the appropriate flasks, 2 ml of this solution was pipetted into three separate, clean, dry test tubes with the labels B, S, and T.
7. Each tube received 1 ml of sulphanilamide and 1 ml of N-naphthyl ethylenediamine diamine solution.
8. The OD of S, T was read against a blank at 540 nm on a spectrophotometer after 20 minutes of vigorously shaking all three tubes.

Table no. VI

Reagents	Test (ml)	Standard (ml)	Blank (ml)
Glycine NaOH buffer	1	1	1
deproteinized sample	1		
standard		1	
distilled water			1
cadmium granules	2.5	2.5	2.5
cadmium granules were swirled once and kept at room temperature for 90 min.			
Distilled water	2	2	2
contents from all these tubes were mixed and a diluted sample was taken in the following tubes			

above diluted sample	2	2	2
sulphanilamide	1	1	1
N-Naphthalin ethylene diamine	1	1	1

After 30 minutes read the optical density at 540 nm

Calculation:

The concentration of Serum NO=$\frac{OD\ of\ Test - OD\ of\ Blank}{OD\ of\ Std - OD\ of\ Blank}$ X 100

=.....mmol/L

9.5. Estimation of Reduced glutathione (GSH)[13]

Blood-reduced glutathione (GSH) was estimated by Earnest Beutler method (Beutler E et al. 1963).

Principle

Red blood cells (RBC) contain non-protein sulphydryl groups in the form of reduced glutathione (GSH). The disulphide molecule 5, 5'-dithiobis-2-nitrobenzoic acid (DTNB) is easily reduced by sulphydryl compounds to a brightly coloured yellow chemical. At 412 nm, optical density was read, and it was shown to be inversely correlated with GSH content.

Reagent

1) Precipitating solution: 1.67gm of glacial metaphosphoric acid, 0.2gm of disodium or dipotassium ethylene diamine tetra acetic acid (EDTA) and 30 gm of sodium chloride was dissolved in 100ml of distilled water.

2) Phosphate solution: 0.3M Na_2HPO_4 (di-sodium hydrogen phosphate) was prepared by dissolving 4.68gm in 100 mL distilled water.

3)1% Sodium citrate: 1gm of sodium citrate was dissolved in 100ml distilled water.

4)5.DTNB reagent: 40mg 5, 5'dithiobis– (2-nitrobenzonic acid) was dissolved in 100ml of 1% sodium citrate.

5) Reduced glutathione standard (0.5 mg/ml): Take 5 mg of reduced glutathione and dissolved in, 10 ml of distilled water.

Procedure

Three test tubes were taken and labeled as blank, standard and test. The procedure of the assay was as given below.

Table no. VII

	Blank	Standard	Test
Whole blood	--	--	0.2 mL
Standard	--	0.4 mL	--
Distilled water	2 mL	1.6Ml	1.8 mL
Mixed well			
Precipitating Solution	3.0 mL	3.0 mL	3.0 mL
Kept for five min	Kept for 5minuutes, centrifuged and 1 ml supernatant was added in a separate labelled test tubes		
Phosphate solution	4.0 mL	4.0 mL	4.0 mL
DTNB Reagen	0.5 mL	0.5 mL	0.5 mL
Mixed and absorbance was read at 412 nm against the blank within 5 minutes			

Calculation

The concentration of GSH=$\frac{OD\ of\ test}{OD\ of\ std}$ X$\frac{con.\ of\ std}{volume\ of\ test}$ X 50

=$\frac{OD\ of\ test}{OD\ of\ std}$ X$\frac{0.04}{0.08}$ X 50

=$\frac{OD\ of\ test}{OD\ of\ std}$ X 50

=……mg/dl

STATISTICAL ANALYSIS:

The data obtained is entered in a Microsoft Excel sheet, and statistical analyses are Performed using a statistical package for the social sciences (SPSS) (Version 20). To check the normality of the data set we have used Kolmogorov-Smirnov and Shapiro-Wilk staistical method. Results are presented as Mean, SD, counts and percentages, and diagrams. For Normally distributed continuous variables to see the correlation between two variables we will use Pearson Correlation coefficient value, the Spearman's rho Correlation coefficient value is used. If more than two groups we will use ANOVA,For not normally distributed ,Kruskal-Walli H Teset. $P<0.05$ will be considered statistically significant. All statistical are performed two-tailed.

RESULT

Table-1 a. Comparison of MMP-9 among Obese, Non-Obese Hypertension and Non- Hypertension individuals

Variables	Group	Mean±Std	Kruskal-Wallis H Test	p-value
MMP-9	Non-obese control	725±258.039	9.80	0.020
	Non obese HTN	866.7±299.471		
	Obese control	820±280.782		
	Obese HTN	977.67±308.278		
	Total	847.87±298.867		

Values are expressed in Mean ± Standard deviation. MMP-9= Matrix metalloproteinase-9, TIMP-1=Tissue inhibitors of matrix metalloprotienase-1, HTN= Hypertension. With p=0.020 shows significant difference.

Table-1.b. Comparison of Tissue inhibitors of metalloproteinase -1 among Obese, Non-Obese Hypertension and Non- Hypertension individuals

Variables	Group	Mean±Std	Kruskal-Wallis H Test	p-value
TIMP-1	Non-obese control	985.7±227.736	8.80	0.032
	Non obese HTN	1236±346.253		
	Obese control	1138.87±227.706		
	Obese HTN	1185.03±366.678		
	Total	1129.66±311.023		

Values are expressed in Mean ± Standard deviation. MMP-9= Matrix metalloprotienase-9, TIMP-1=Tissue inhibitors of matrix metalloprotienase-1, HTN= Hypertension. With p=0.032 shows significant difference.

2. Correlation of Anthropometric, Physiological and Biochemical parameters with Matrix metalloproteinase -9 and Tissue inhibitors of metalloproteinase -1 in Non-obese control group

We found non-significant positive correlation between Matrix metalloproteinase -9 and Body mass index(r=0.001, p=0.995). There is a positive correlation between **Tissue inhibitors of metalloproteinase -1** and and Body mass index (r=0.177, p=0.339)

Correlation between BMI and MMP-9

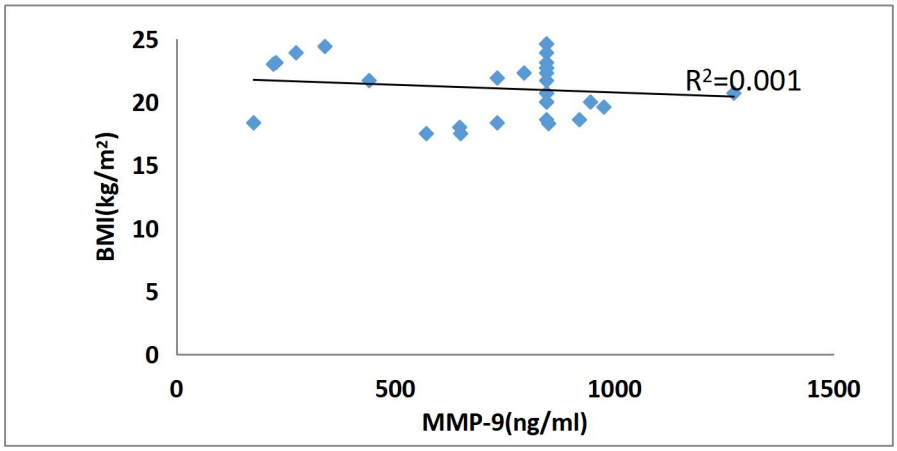


Fig. No.- 2.a non-significant positive correlation between Matrix metalloproteinase -9 and Body mass index with p>0.05

Correlation between BMI and TIMP-1

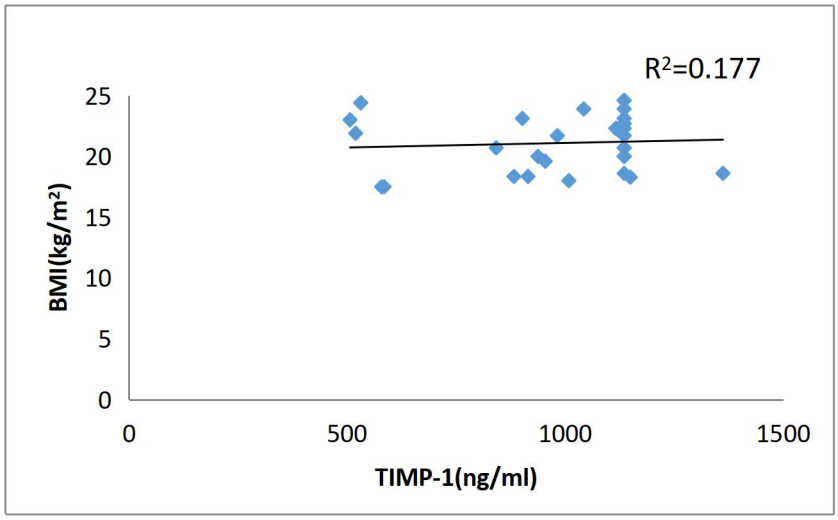


Fig. No.- 2.b positive correlation between Tissue inhibitors of metalloproteinase -1 and and Body mass index with p>0.05

Correlation of Matrix metalloproteinase -9 and Tissue inhibitors of metalloproteinase -1 with Physiological parameters in Non-obese control

We found non-significant positive correlation between Matrix metalloproteinase -9 and Systolic blood pressure(r=0.104, p=0.576),Diastolic blood pressure (r=0.146, p=0.432), Mean arterial presuure (r=0.099,p=0.595). Non-significant positive correlation between TIMP-1 and Systolic blood pressure(r=0.230, p=0.213) MAP(r=0.325, p=0.075), significant positive correlation between TIMP-1 and Diastolic blood pressure (r=0.395, p=0.028).

Correlation between SBP and MMP-9

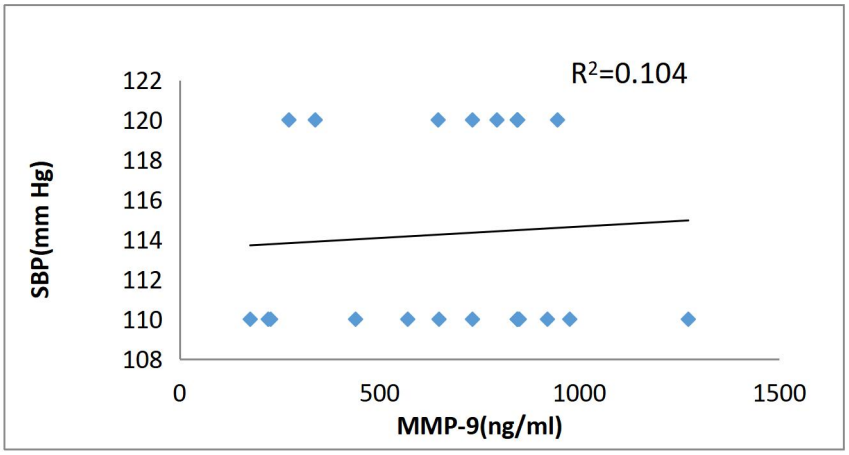


Fig. No.- 2.c We found non-significant positive correlation between Matrix metalloproteinase -9 and Systolic blood pressure with p=0.576)

Correlation between SBP and TIMP-1

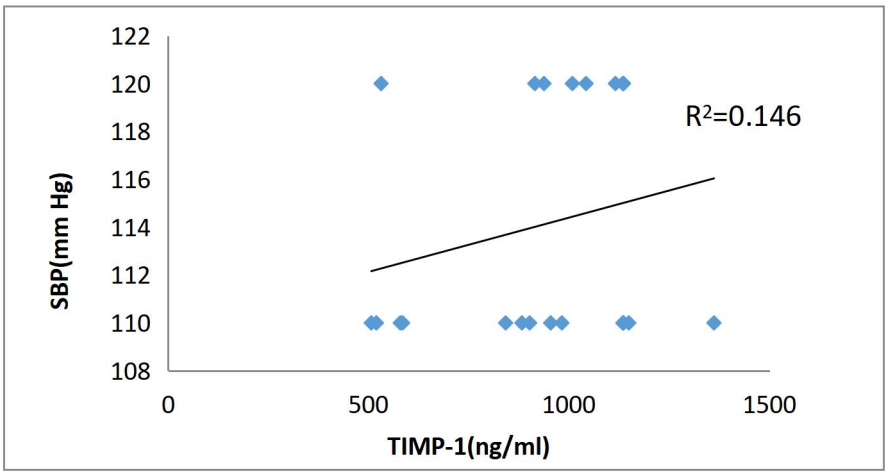


Fig. No.- 2.d Non-significant positive correlation between TIMP-1 and Systolic blood pressure(r=0.230, p=0.213)

Correlation between DBP and MMP-9

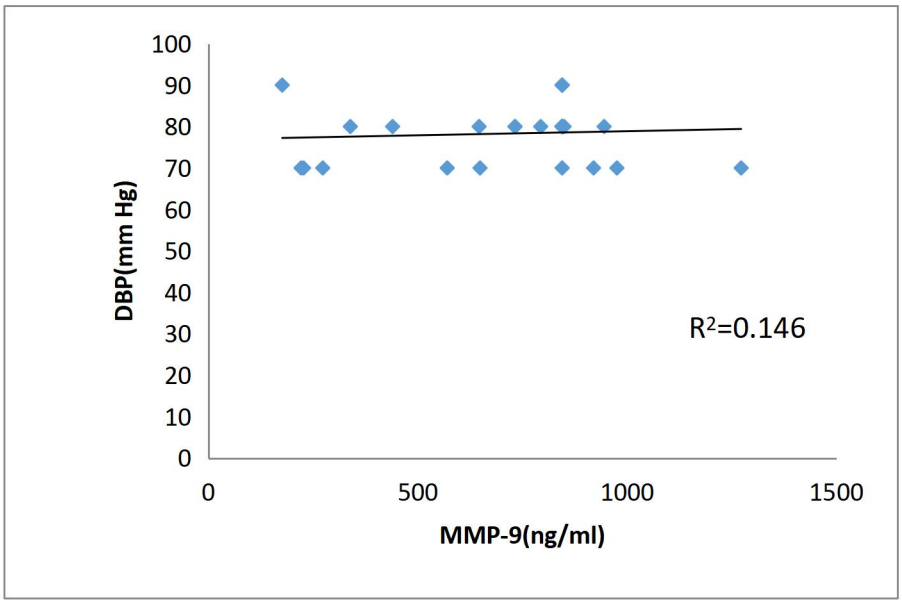


Fig. No.- 2.e :non-significant positive correlation between Matrix metalloproteinase -9 Diastolic blood pressure with p>0.05

Correlation between DBP and TIMP-1

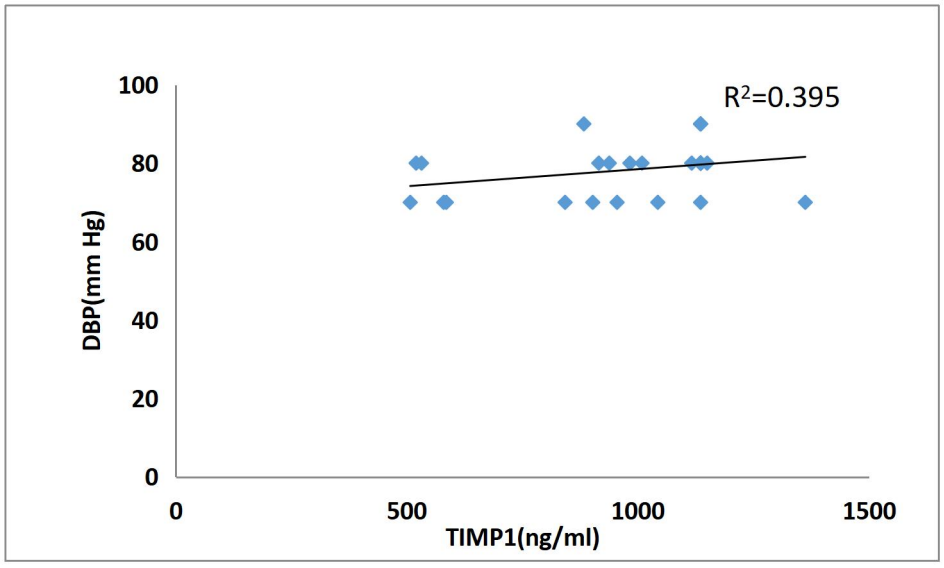


Fig. No.- 2.f :significant positive correlation between TIMP-1 and Diastolic blood pressure with p>0.05

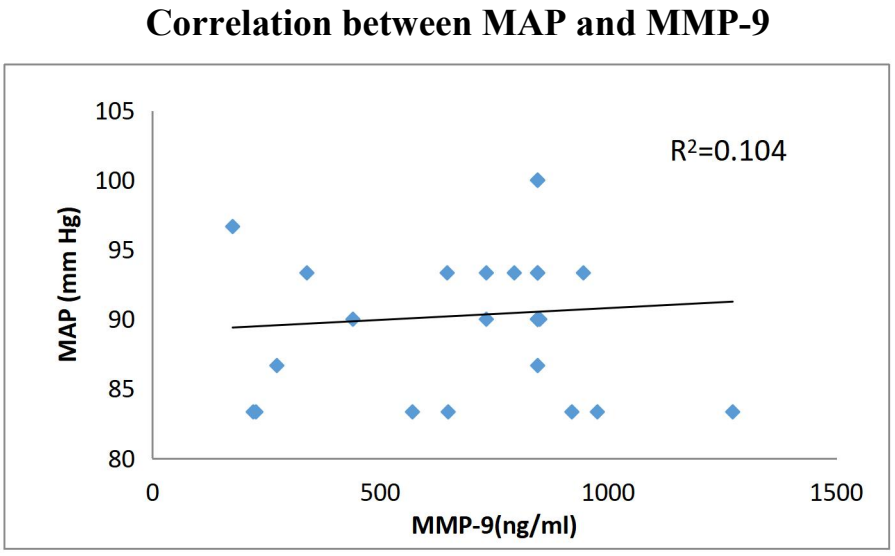


Fig. No.- 2.g : non-significant positive correlation between Matrix metalloproteinase -9 and Mean arterial presuure with p>0.05

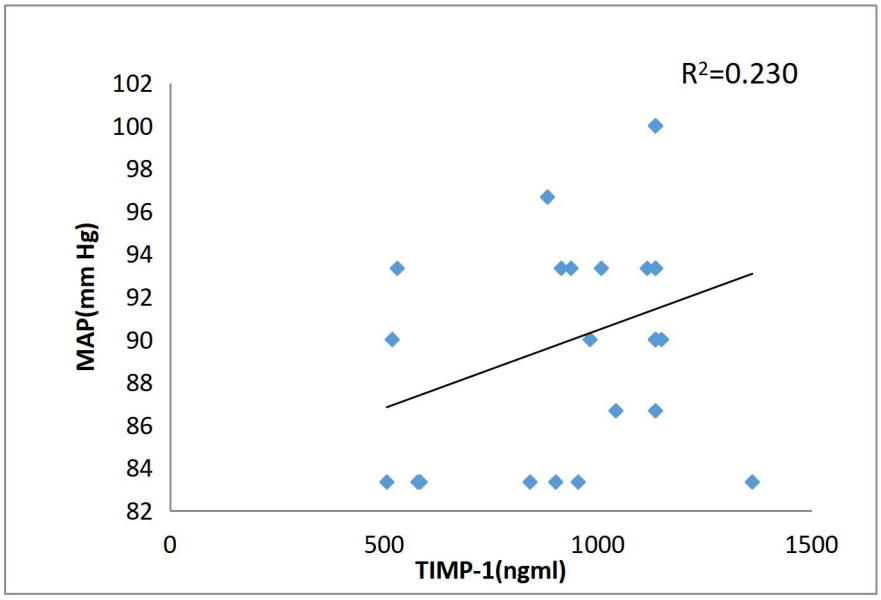


Fig. No.- 2.h : Non-significant positive correlation between TIMP-1 and MAP with p>0.05

Correlation with Biochemical parameters

Correlation of Matrix metalloproteinase -9 with Tissue inhibitors of metalloproteinase -1 in Non-obese control

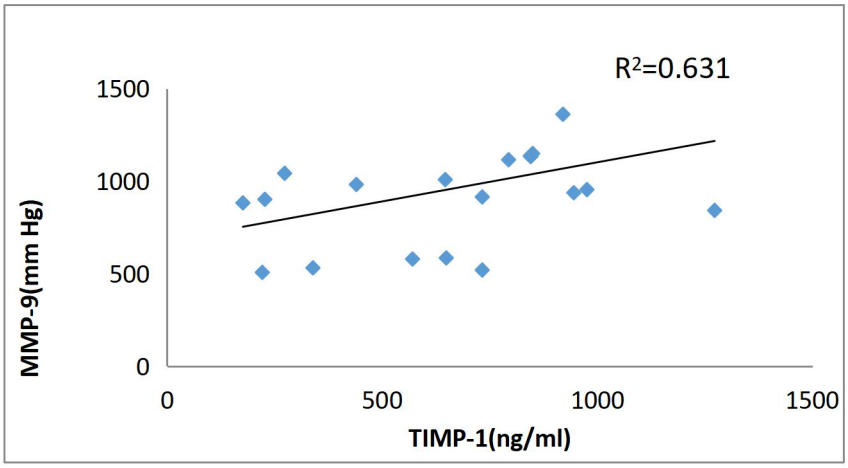


Fig .No.-2.I : Significant positive correlation between Matrix metalloproteinase -9 and Tissue inhibitors of metalloproteinase -1 with $p>0.05$

Correlation of Matrix metalloproteinase -9 and Tissue inhibitors of metalloproteinase -1 with Oxidative stress parameters in Non-obese control

There is a non significant negative correlation Matrix metalloproteinase -9 and MDA(r= -0.049, p=0.769), Reduced glutathione (r= - 0.008, p=0.967), non significant positive correlation between Matrix metalloproteinase -9 and NO (r=0.106 p=0.571) There is non-significant positive correlation between Tissue inhibitors of metalloproteinase -1 and Malondialdehyde (r= 0.072, p=0.705) Nitric oxide (r= 0.066, p=0.726) and non significant negative correlation between Tissue inhibitors of metalloproteinase -1 and Reduced glutathione (r= -0.147, p= 0.431)

j.Correlation between MDA and MMP-9

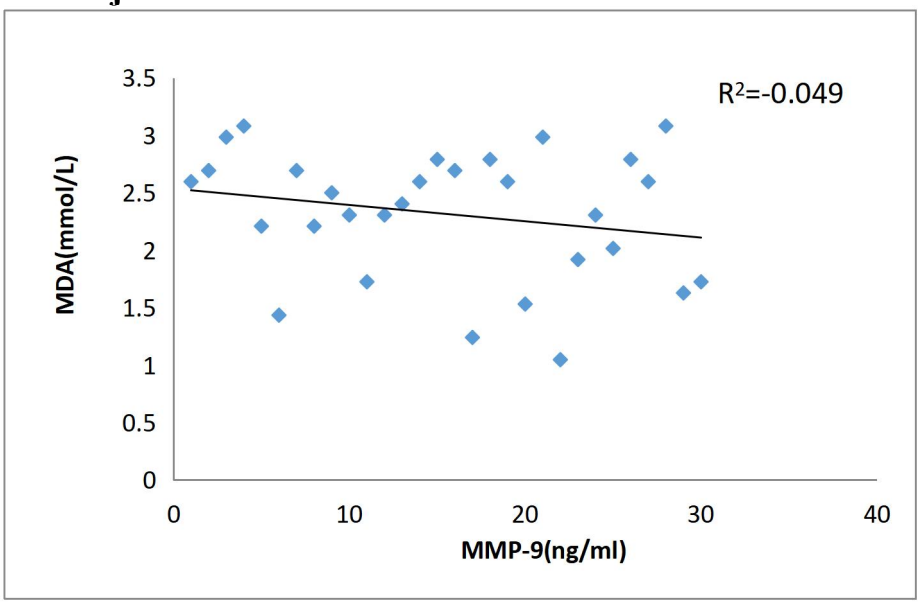


Fig .No.-2.j : Non significant negative correlation Matrix metalloproteinase -9 and MDA with p>0.05

k .Correlation between MDA and TIMP-1

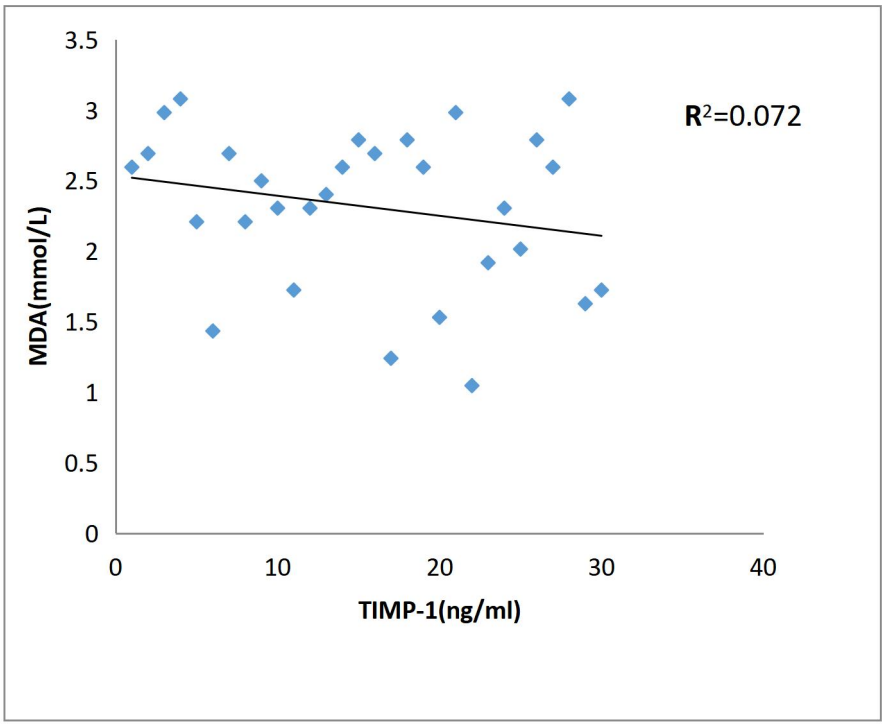


Fig .No.-2.k : Non-significant positive correlation between Tissue inhibitors of metalloproteinase -1 and Malondialdehyde with p>0.05

l.Correlation between GSH and MMP-9

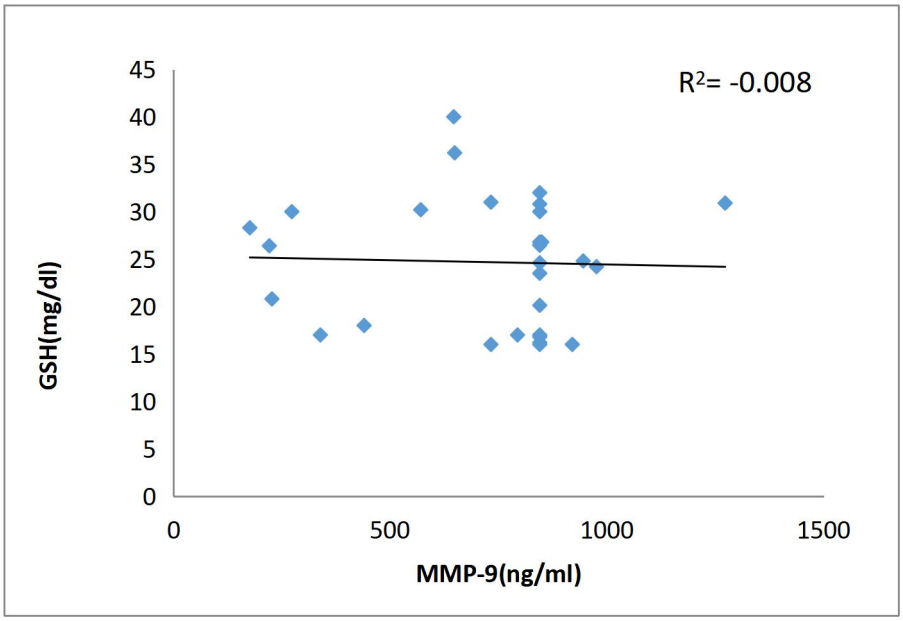


Fig .No.-2.l : Non significant negative correlation Matrix metalloproteinase -9 and Reduced glutathione with p>0.05

m.Correlation between GSH and TIMP-1

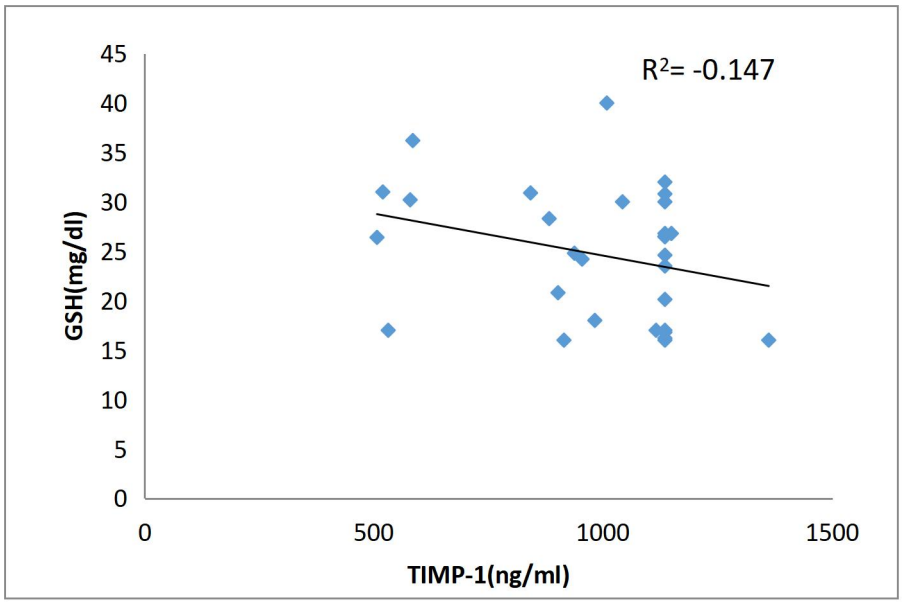


Fig .No.-2.m: Non significant negative correlation between Tissue inhibitors of metalloproteinase -1 and Reduced glutathione with p>0.05

n.Correlation between NO and MMP-9

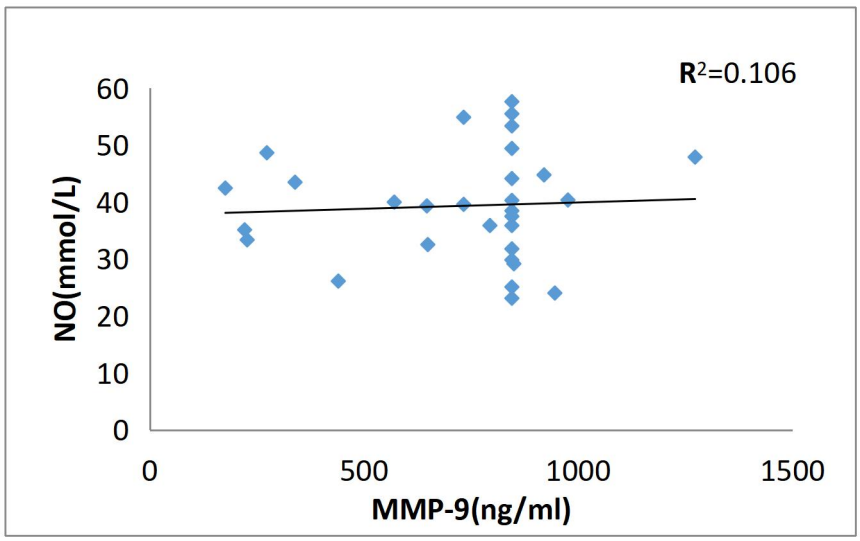


Fig .No.-2.n : Non significant positive correlation between Matrix metalloproteinase -9 and NO with p>0.05

o. Correlation between NO and TIMP-1

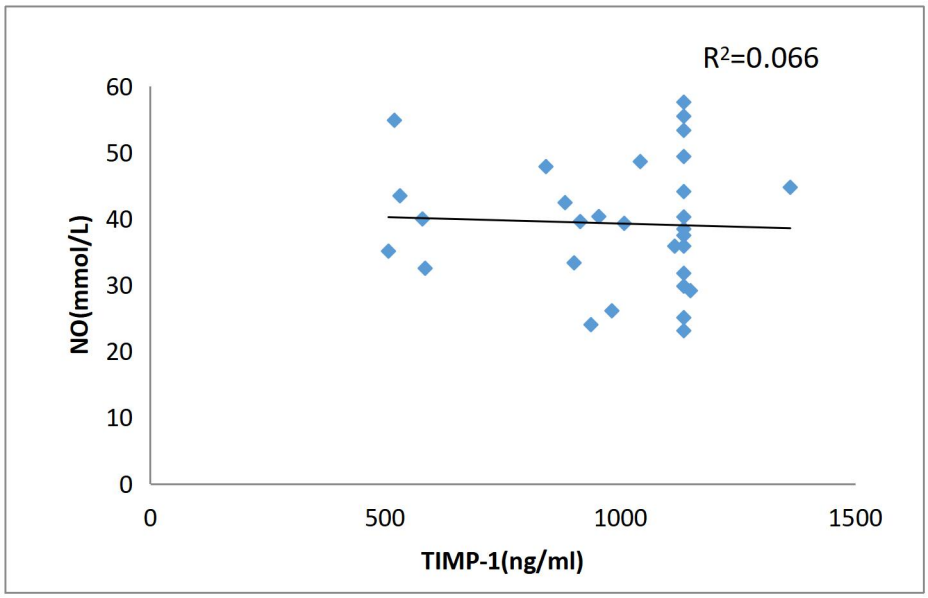


Fig .No.-2.o: Non-significant positive correlation between Tissue inhibitors of metalloproteinase -1 Nitric oxide with p>0.05

3.Correlation of Anthropometric, Physiological and Biochemical parameters with Matrix metalloproteinase -9 and Tissue inhibitors of metalloproteinase -1 in non-obese Hypertension group.

We found non-significant positive correlation between Body mass index (r= 0.040, p=0.855) and Matrix metalloproteinase -9 . There is a non significant negative Body mass index(r=- 0.051, p=0.818) with Tissue inhibitors of metalloproteinase -1 respectively.

a .Correlation between BMI and MMP-9

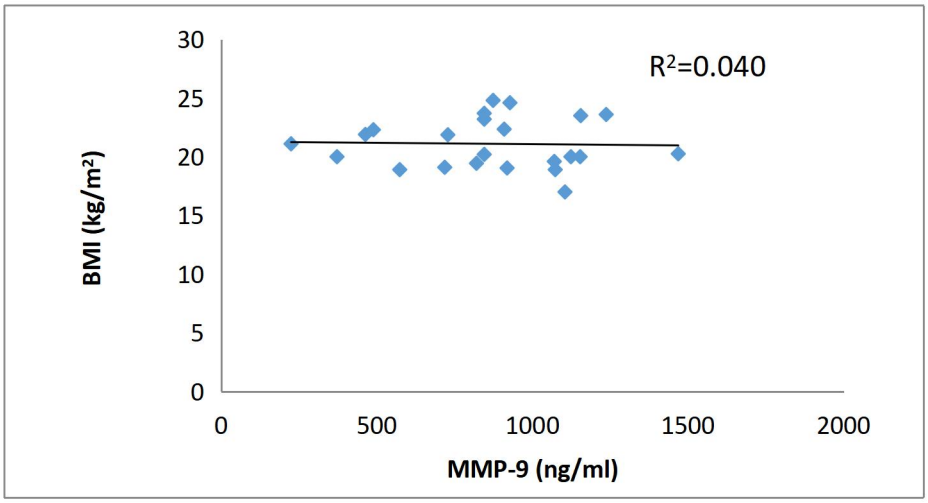


Fig .No.-3.a : Non-significant positive correlation between Body mass index and Matrix metalloproteinase -9 with p>0.05

b .Correlation between BMI and TIMP-1

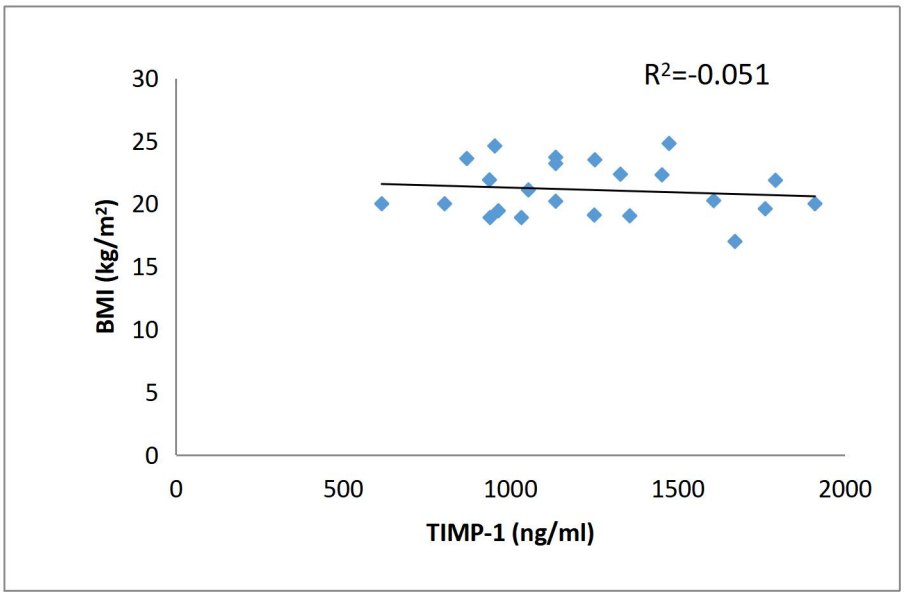


Fig .No.-3.b : Non significant negative Body mass index with Tissue inhibitors of metalloproteinase -1 respectively with p>0.05

Correlation of Matrix metalloproteinase -9 and Tissue inhibitors of metalloproteinase -1 with Physiological parameters in Non-obese Hypertension

There is a non significant positive correlation between Matrix metalloproteinase -9 and Systolic blood pressure (r=0.050 p=0.819) non-significant negative correlation between Diastolic blood pressure (r= -0.314, p=0.144), Mean arterial presuure(r=-0.259, p==0.233). There is a non significant positive correlation between Tissue inhibitors of metalloproteinase -1 and Diastolic blood pressure (r= 0066 p=0.766) non significant negative correlation of Systolic blood pressure(r=-0.230, p=0.219), Mean arterial presuure (R=-0.056, P=0.798)

c .Correlation between SBP and MMP-9

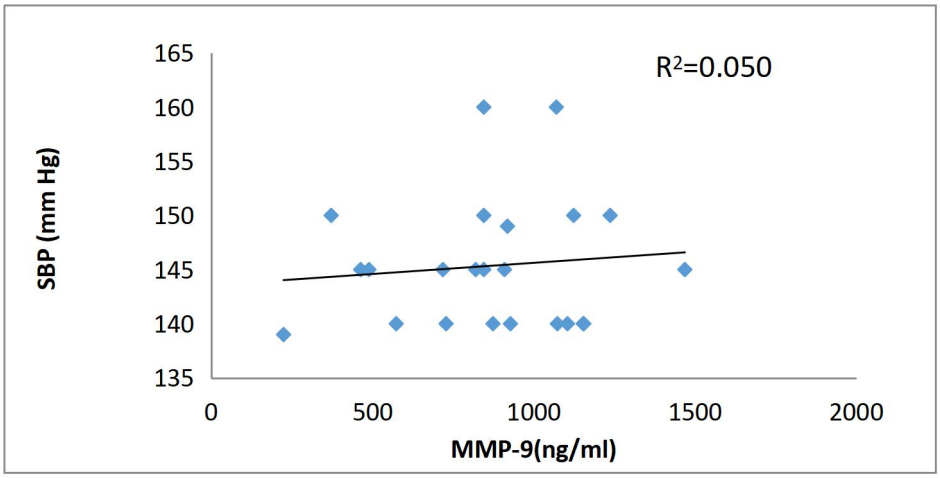


Fig.no-3.c : Non significant positive correlation between Matrix metalloproteinase -9 and Systolic blood pressure with p>0.05

d. correlation between SBP and TIMP-1

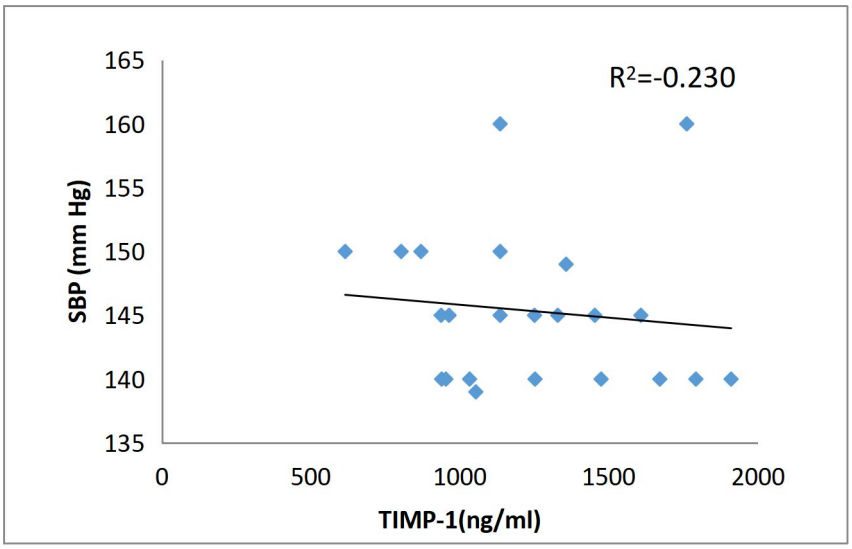


Fig.no-3.d : non significant negative correlation between Tissue inhibitors of metalloproteinase -1 and Systolic blood pressure with p>0.05

e. correlation between DBP and MMP-9

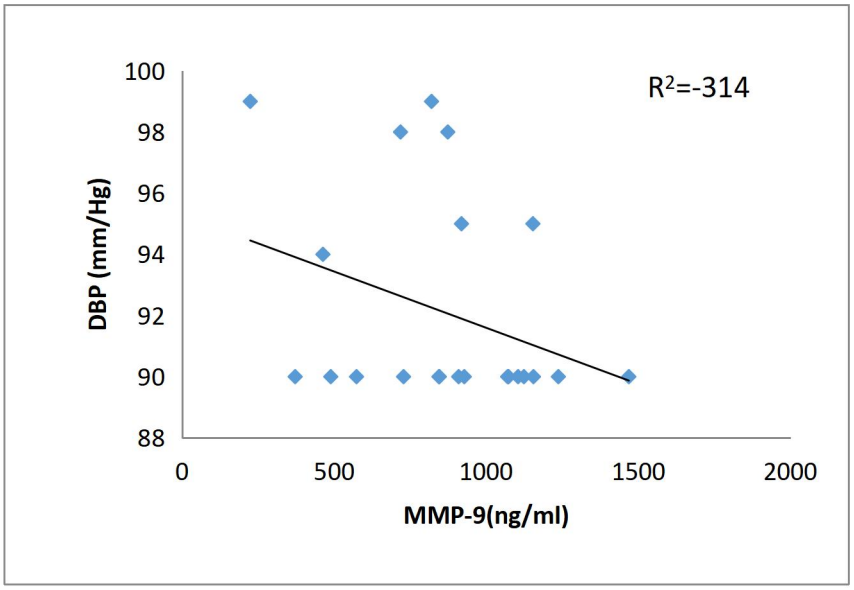


Fig.no-3.e : non-significant negative correlation between Diastolic blood pressure and Matrix metalloproteinase -9 with p>0.05

f .Correlation between DBP and TIMP-1

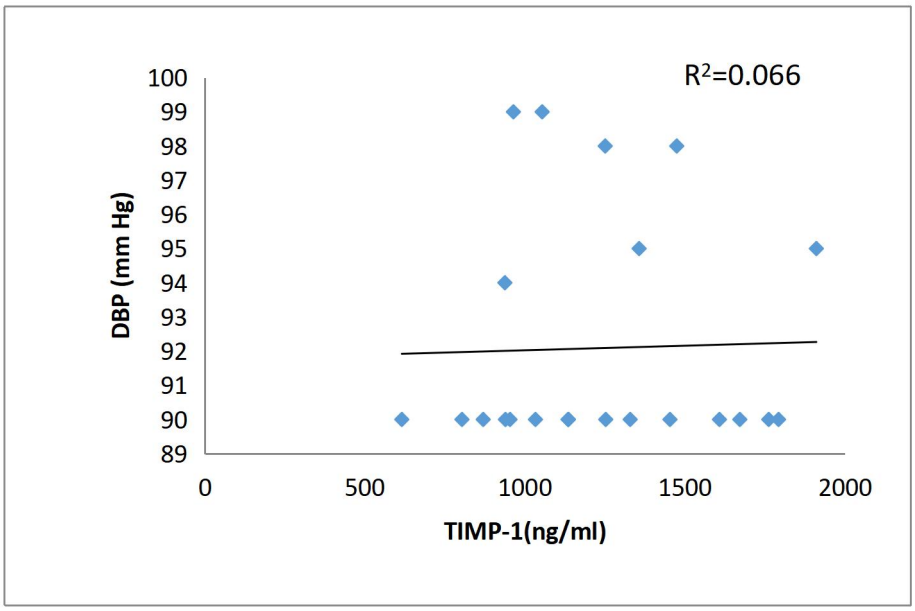


Fig.no-3.f : There is a non significant positive correlation between Tissue inhibitors of metalloproteinase -1 and Diastolic blood pressure with p>0.05

g. **Correlation between MAP and MMP-9**

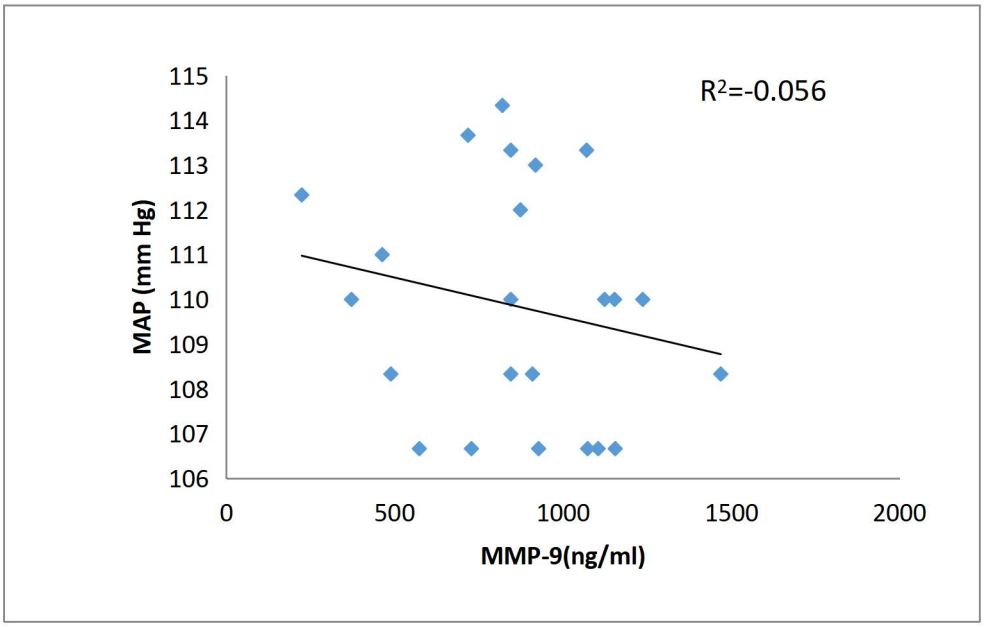


Fig.no-3.g: non-significant negative correlation between Matrix metalloproteinase -9 and Mean arterial pressure with p>0.05

h. Correlation between MAP and TIMP-1

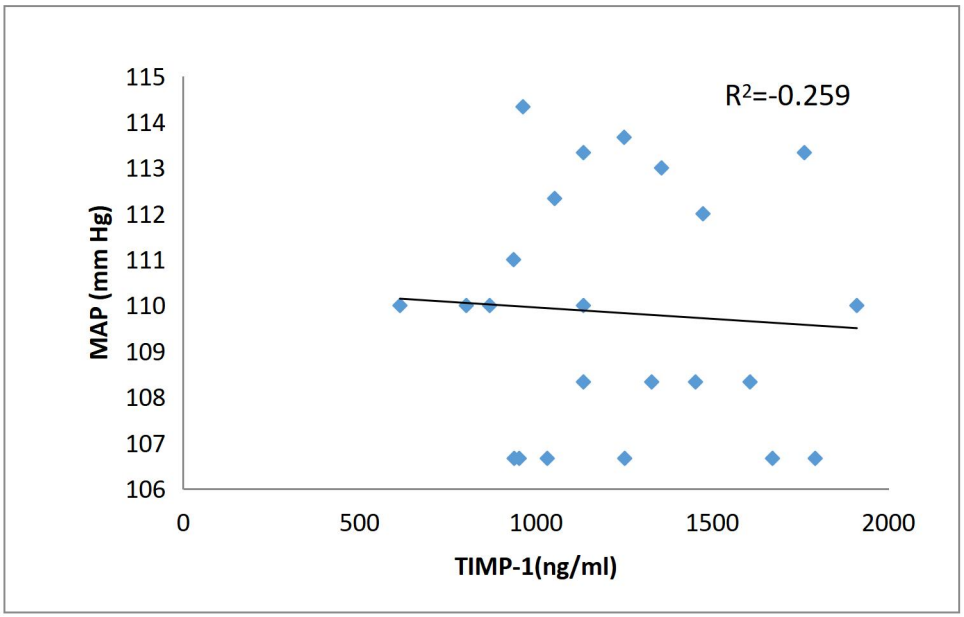


Fig.no-3.h : Non significant negative correlation between Tissue inhibitors of metalloproteinase -1 and Mean arterial presuure with p>0.05

Correlation with Biochemical parameters

Tabble-3.3.Correlation of Matrix metalloproteinase -9 with Tissue inhibitors of metalloproteinase -1 in Non-obese Hypertension

We found non-significant positive correlation between Matrix metalloproteinase -9 (r=0.219, p=0.316) and Tissue inhibitors of metalloproteinase -1 in non-obese **Hypertension** individuals.

i.Correlation of MMP-9 with TIMP-1

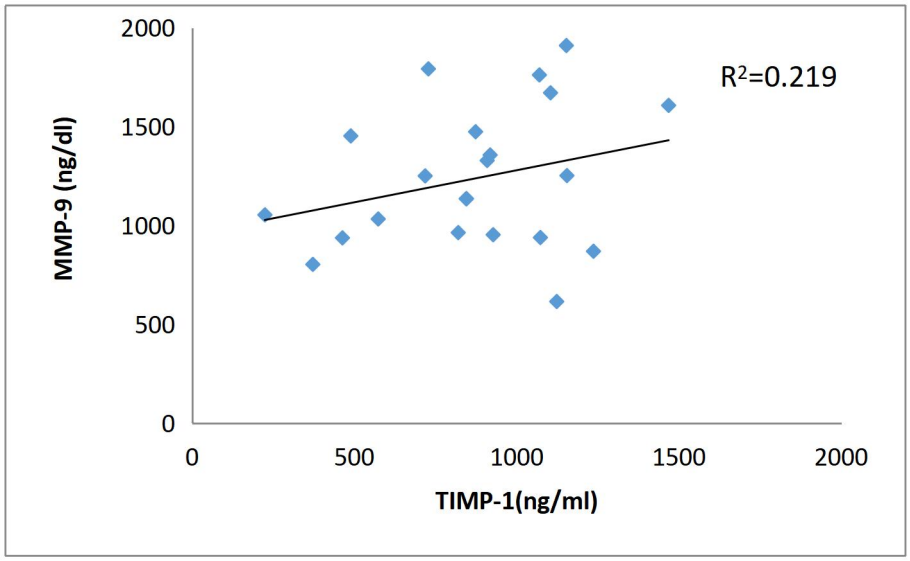


Fig.no-3.I:non-significant positive correlation between Matrix metalloproteinase -9 and Tissue inhibitors of metalloproteinase -1 with p>0.05

Correlation of MMP-9 and TIMP-1 with oxidative stress parameters in Non-obese Hypertension

We found non-significant negative correlation between Matrix metalloproteinase -9 and Malondialdehyde , Reduced glutathione, Nitric oxide, Duration of Hypertension, Duration of treatment. There is a non-significant negative correlation between Tissue inhibitors of metalloproteinase -1 and Malondialdehyde , Nitric oxide, and non significant positive correlation between Tissue inhibitors of metalloproteinase -1 and Reduced glutathione, Duration of Hypertension, Duration of treatment.

j.Correlation between MDA and MMP-9

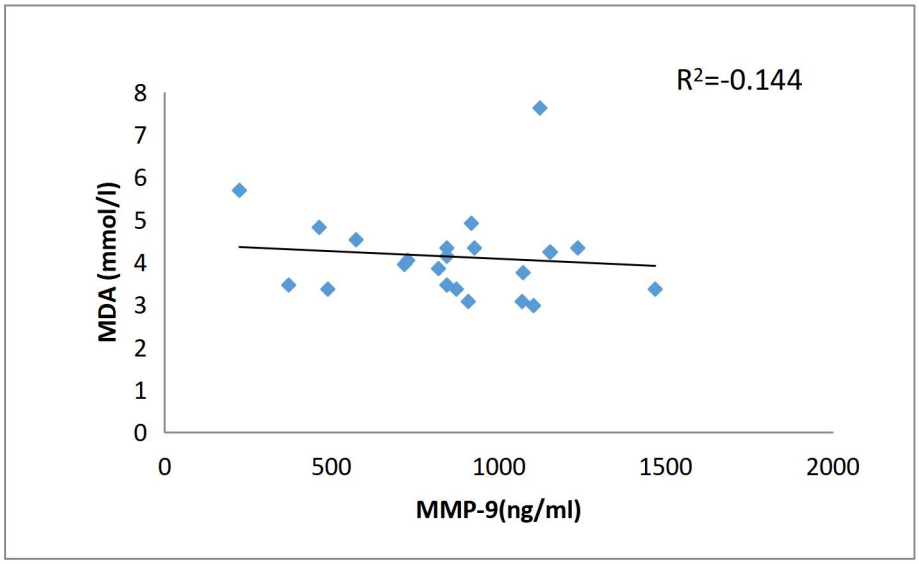


Fig.no-3.j: non-significant negative correlation between Matrix metalloproteinase -9 and Malondialdehyde with p>0.05

k.Correlation between MDA and TIMP-1

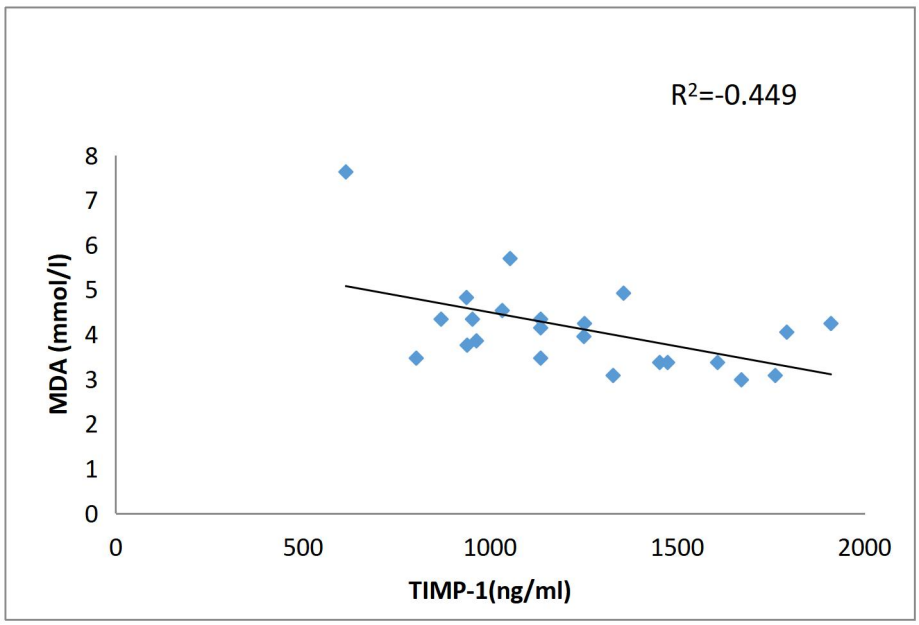


Fig.no-3.k: There is a non-significant negative correlation between Tissue inhibitors of metalloproteinase -1 and Malondialdehyde with p>0.05

l.Correlation between GSH and MMP-9

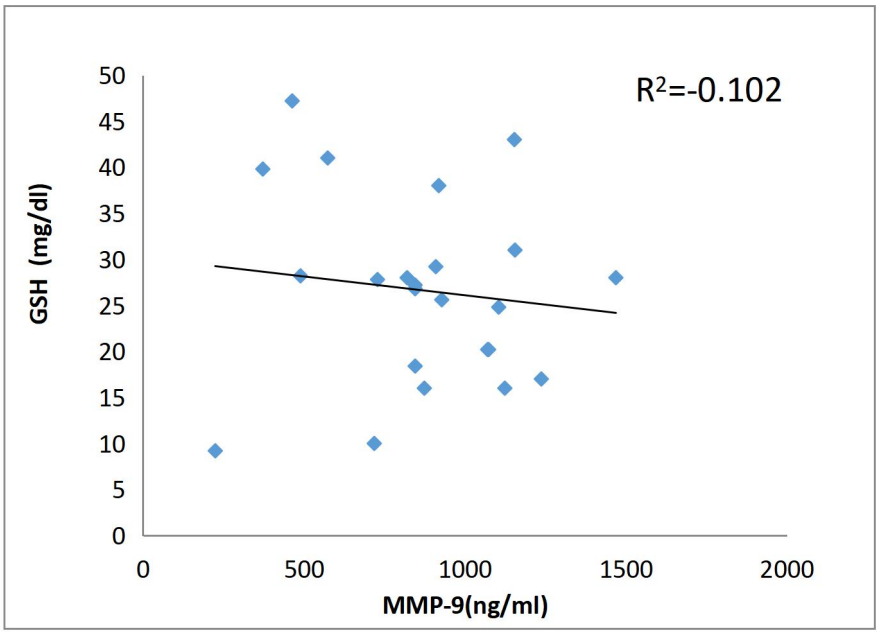


Fig.no-3.l: non-significant negative correlation between Matrix metalloproteinase -9 and Reduced glutathione with p>0.05

m.Correlation between GSH and TIMP-1

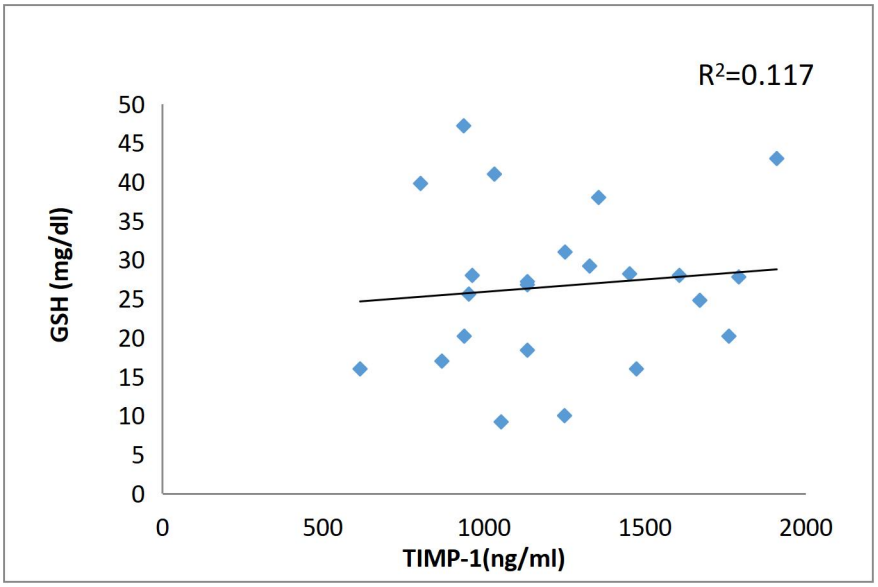


Fig.no-3.m non significant positive correlation between Tissue inhibitors of metalloproteinase -1 and Reduced glutathione with p>0.05

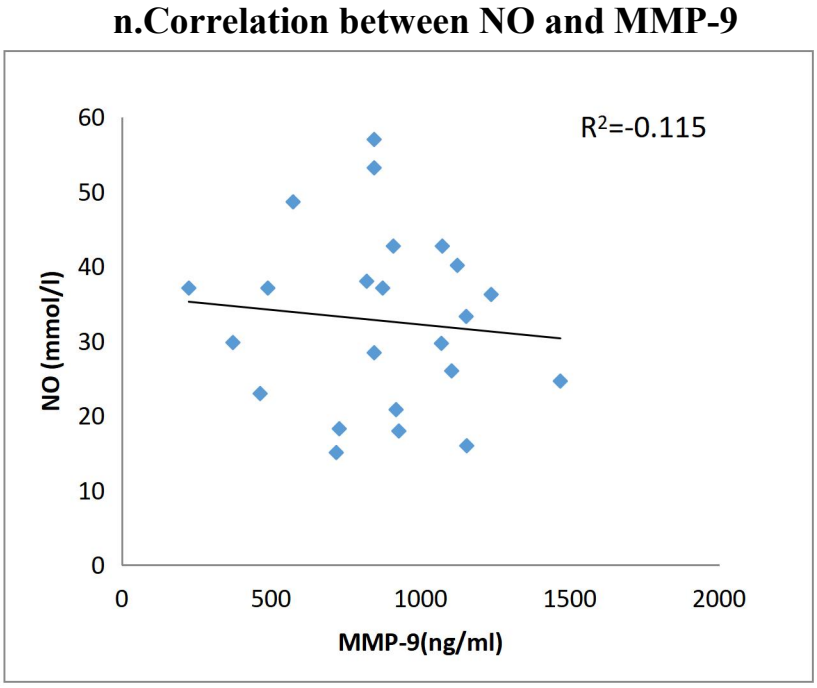


Fig.no-3.n: non-significant negative correlation between Matrix metalloproteinase -9 and Nitric oxide with p>0.05

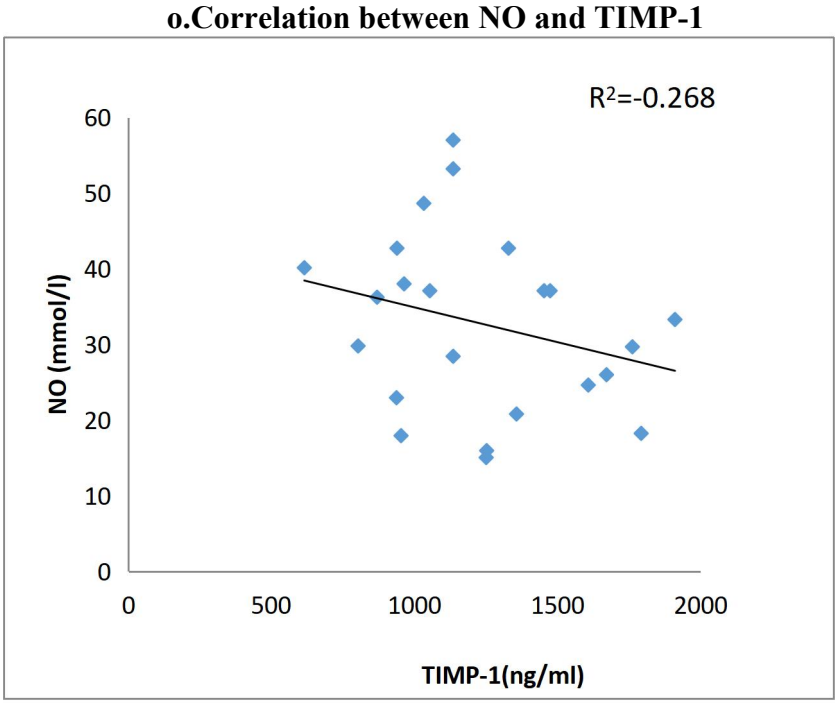


Fig.no-3.o: There is a non-significant negative correlation between Tissue inhibitors of metalloproteinase -1 and Nitric oxide with p>0.05

4.Correlation of Anthropometric Physiological and Biochemical parameters with Matrix metalloproteinase -9 and Tissue inhibitors of metalloproteinase -1 in obese control group

Correlation of Matrix metalloproteinase -9 and Tissue inhibitors of metalloproteinase -1 with Anthropometric parameters in obese control

We found non-significant positive correlation between Matrix metalloproteinase -9 and Body mass index(r= 0061, p=0.781). There is non-significant negative correlation between Tissue inhibitors of metalloproteinase -1 and Body mass index(r=-0.781, p=0.415)

a.Correlation between BMI and MMP-9

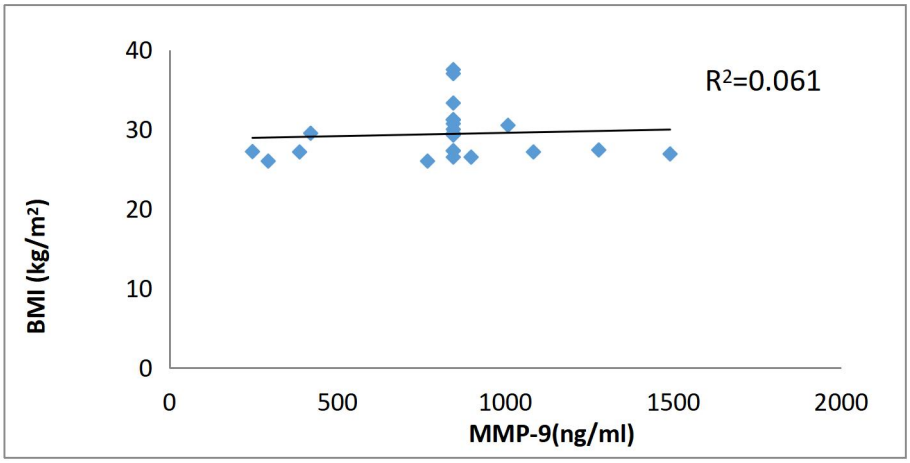


Fig.no-4.a: non-significant positive correlation between Matrix metalloproteinase -9 and Body mass index with p>0.05

b. Correlation between BMI and TIMP-1

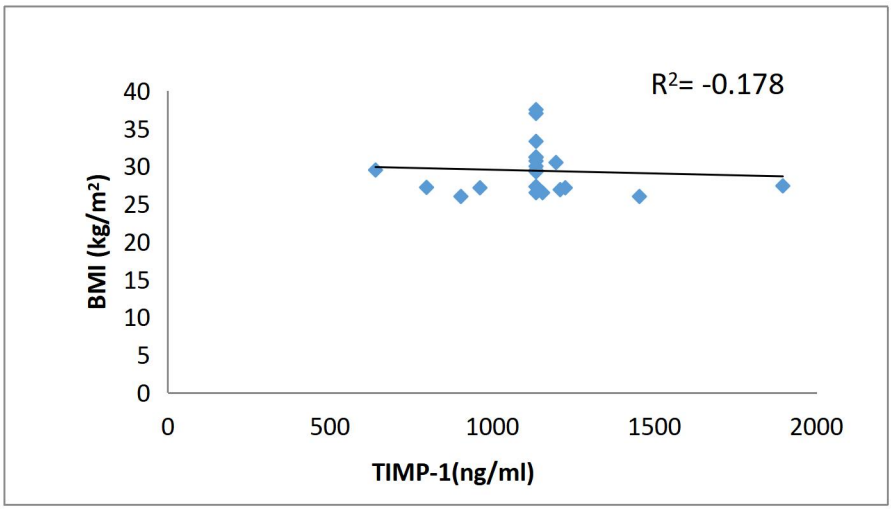


Fig.no-4.b : There is non-significant negative correlation between Tissue inhibitors of metalloproteinase -1 and Body mass index with p>0.05

Correlation of Matrix metalloproteinase -9 and Tissue inhibitors of metalloproteinase -1 with Physiological parameters in obese control

There is a non significant positive correlation between Systolic blood pressure (r=0.157, p=0.474), non significant negative correlation between Diastolic blood pressure (r=-0.180, p=0.142), Mean arterial presuure (r=0.090, p=0.684) and Matrix metalloproteinase -9 . And there is a non significant positive correlation between Systolic blood pressure(r=0.047, p=0.831) and non significant negative correlation between Diastolic blood pressure (r=-0.267, p=0.218), Mean arterial presuure (r=-0.217, p=0.320) and Tissue inhibitors of metalloproteinase -1.

c.Correlation between SBP and Matrix metalloproteinase -9

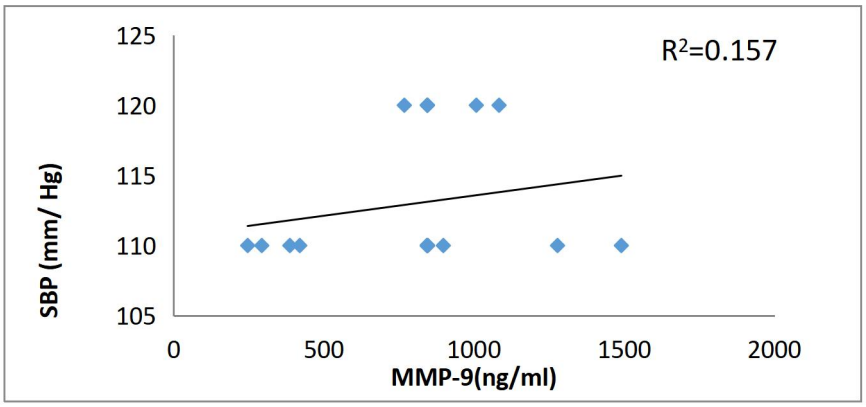


Fig.no-4.c: There is a non significant positive correlation between Systolic blood pressure and Matrix metalloproteinase -9 with p>0.05

d. Correlation between SBP and TIMP-1

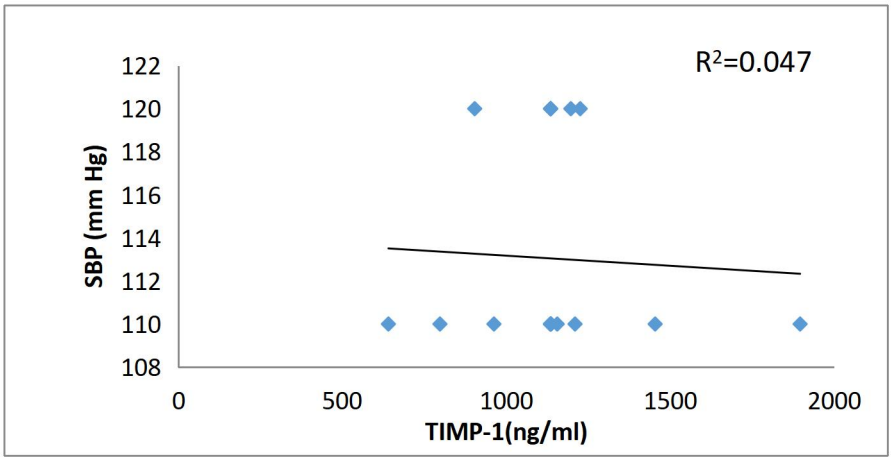


Fig.no-4.d : And there is a non significant positive correlation between Systolic blood pressure(r=0.047, p=0.831) and Tissue inhibitors of metalloproteinase -1 with p>0.05

e .Correlation between DBP and MMP-9

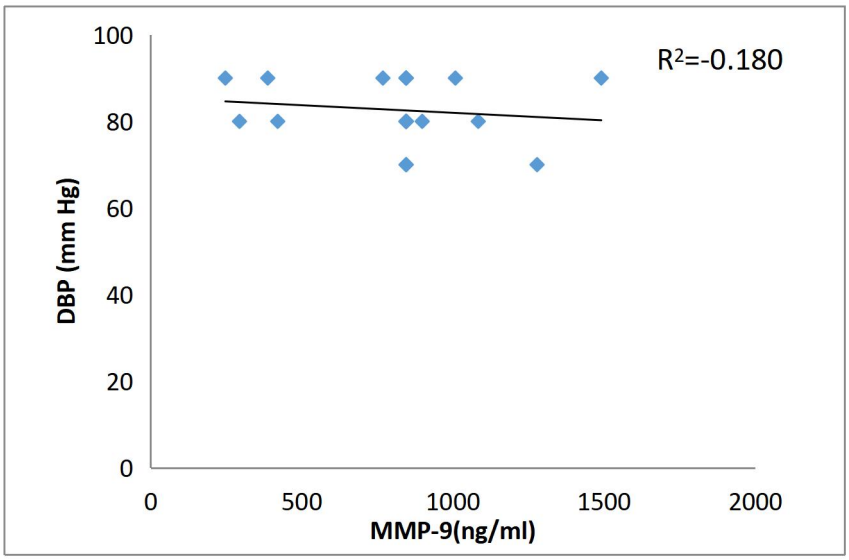


Fig.no-4.e : non significant negative correlation between Diastolic blood pressure and Matrix metalloproteinase -9 with p>0.05

f. Correlation between DBP and TIMP-1

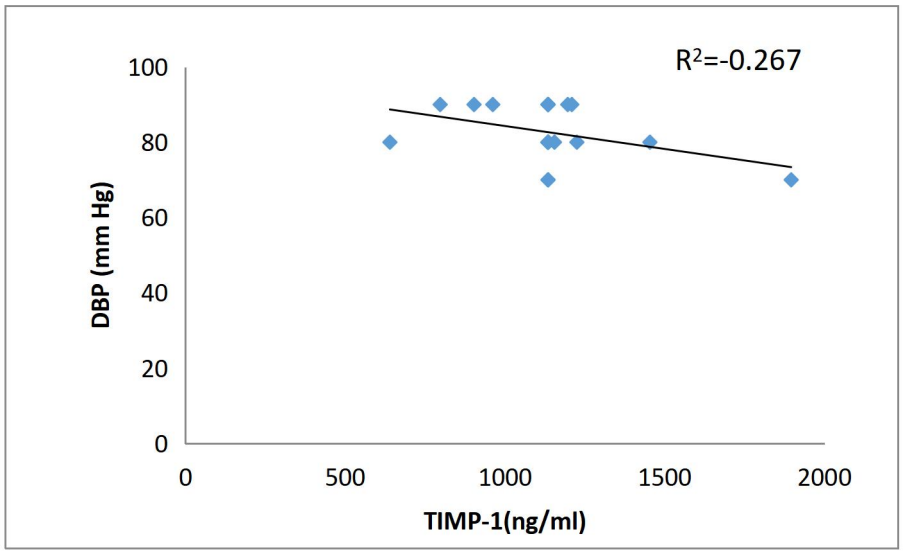


Fig.no-4.f : non significant negative correlation between Diastolic blood pressure and Tissue inhibitors of metalloproteinase -1 with p>0.05

g .Correlation between MAP and MMP-9

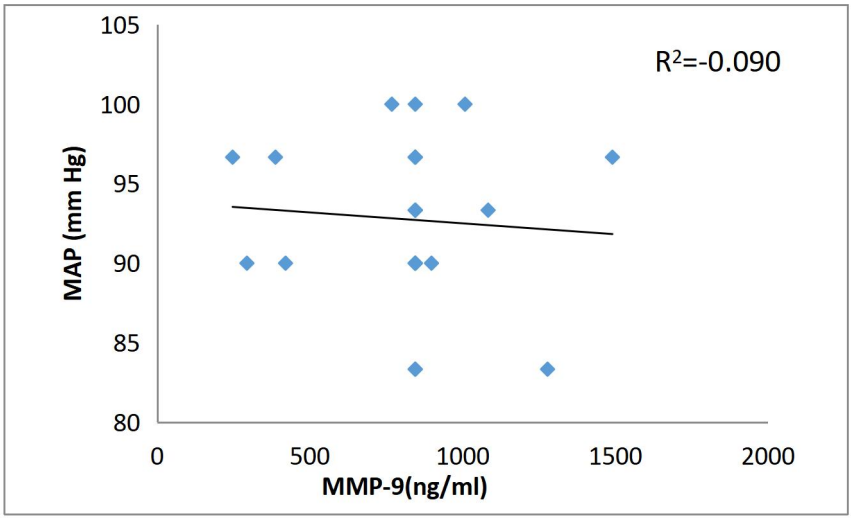


Fig.no-4.g : non significant negative correlation between Mean arterial pressure and MMP-9 with p>0.05

h. Correlation between MAP and TIMP-1

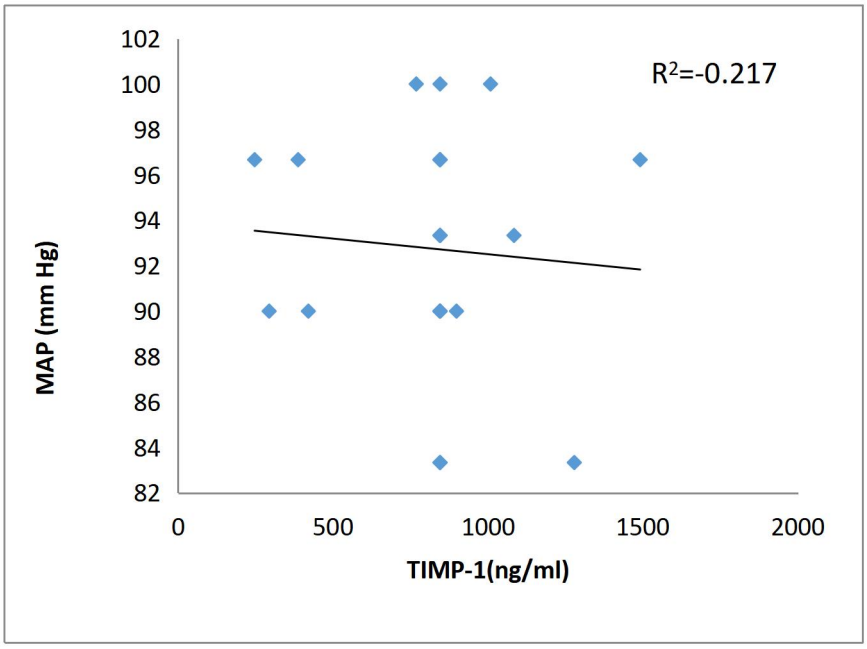


Fig.no-4.h : non significant negative correlation between Mean arterial pressure and Tissue inhibitors of metalloproteinase -1.with p>0.05

Correlation of Matrix metalloproteinase -9 with Tissue inhibitors of metalloproteinase -1 in obese control

We found significant positive correlation between Matrix metalloproteinase -9 and Tissue inhibitors of metalloproteinase -1 in obese control individual (r=0.735, p-0.000)

i.Correlation of MMP-9 with TIMP-1

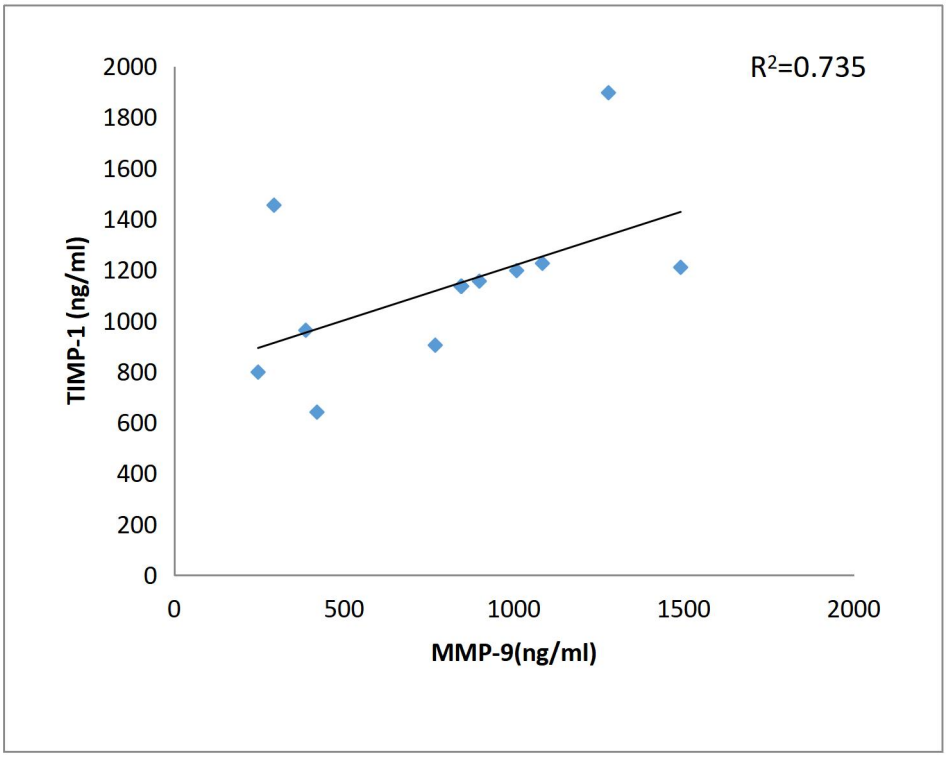


Fig.no-4.I : Significant positive correlation between Matrix metalloproteinase -9 and Tissue inhibitors of metalloproteinase -1 with p=0.000

Correlation of Matrix metalloproteinase -9 and TIMP-1 with oxidative stress parameters in Obese control

There is a significant positive correlation between Matrix metalloproteinase -9 and Reduced glutathione (r=0.404, p=0.056) , non significant positive correlation between Malondialdehyde (r=0.226, p=0.299) and non significant negative correlation between NO(r=-0.133 p=0.545) and Matrix metalloproteinase -9 . Significant positive correlation between Tissue inhibitors of metalloproteinase -1 and Reduced glutathione (r=0.531, p=0.009) Non significant positive correlation between Malondialdehyde (r=0.196, p=0.371), NO(r=0.088, p=0.689) .

j.Correlation between MDA and MM-9

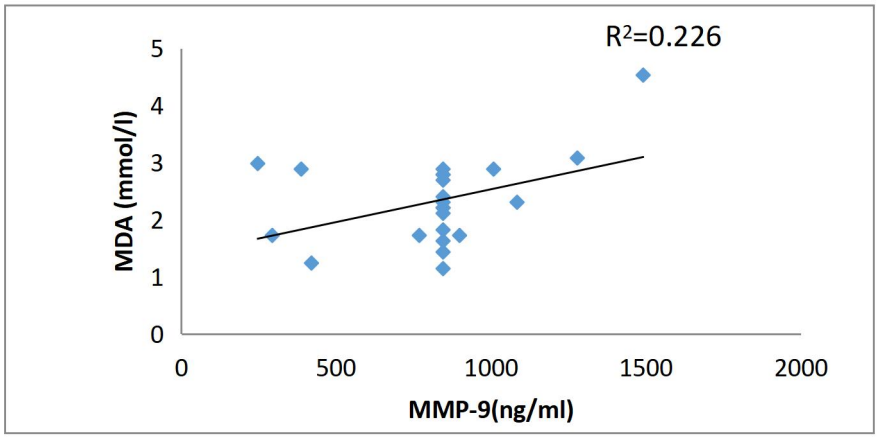


Fig.no-4.j: Non significant positive correlation between Malondialdehyde and Matrix metalloproteinase -9 with p> 0.05

k.Correlation between MDA and TIMP-1

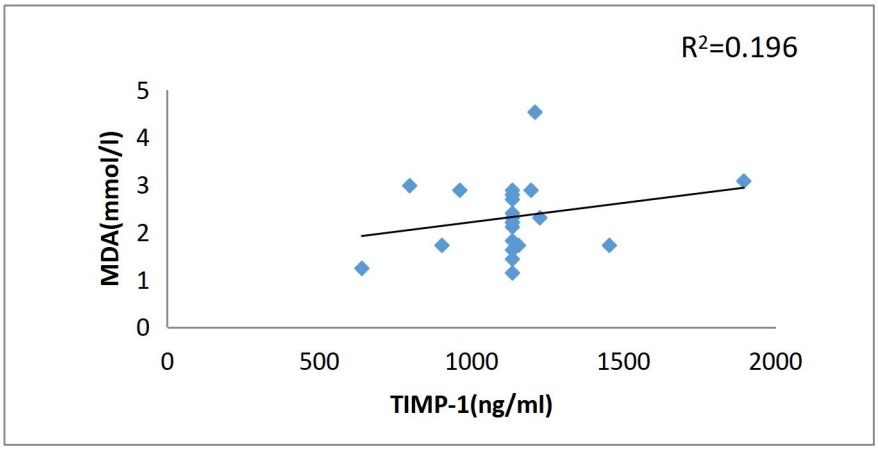


Fig.no-4.k : Non significant positive correlation between Malondialdehyde and Tissue inhibitors of metalloproteinase -1 with p> 0.05

l.Correlation between GSH and MMP-9

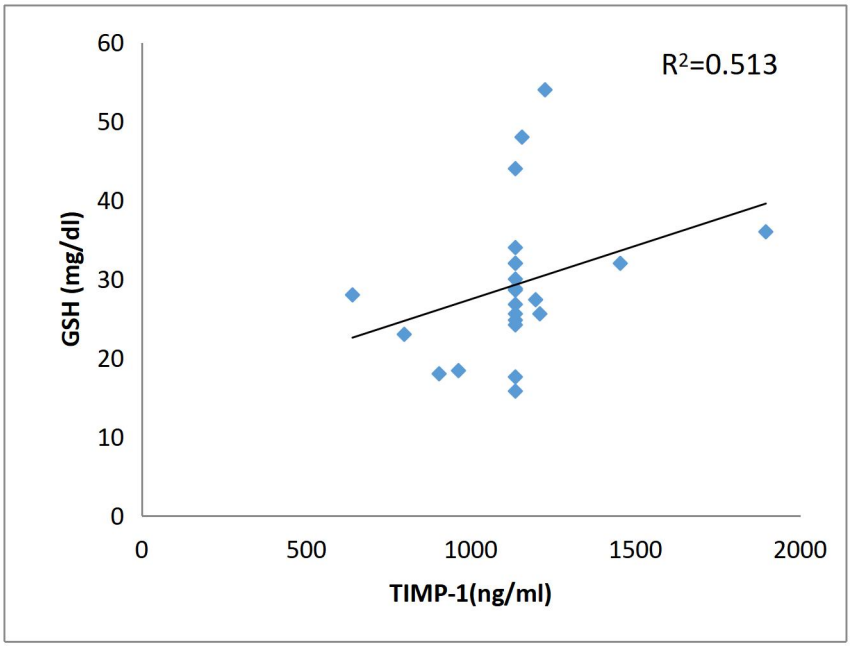


Fig.no-4.l : There is a significant positive correlation between Matrix metalloproteinase -9 and Reduced glutathione with **p=0.05**

m.Correlation between GSH and TIMP-1

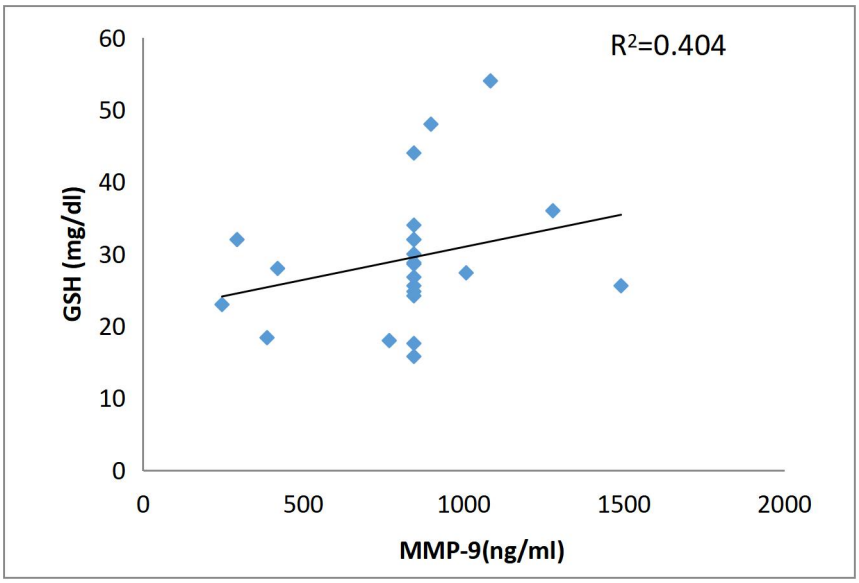


Fig.no-4.m : Significant positive correlation between Tissue inhibitors of metalloproteinase -1 and Reduced glutathione with **p=0.009**

n.Correlation between NO and MMP-9

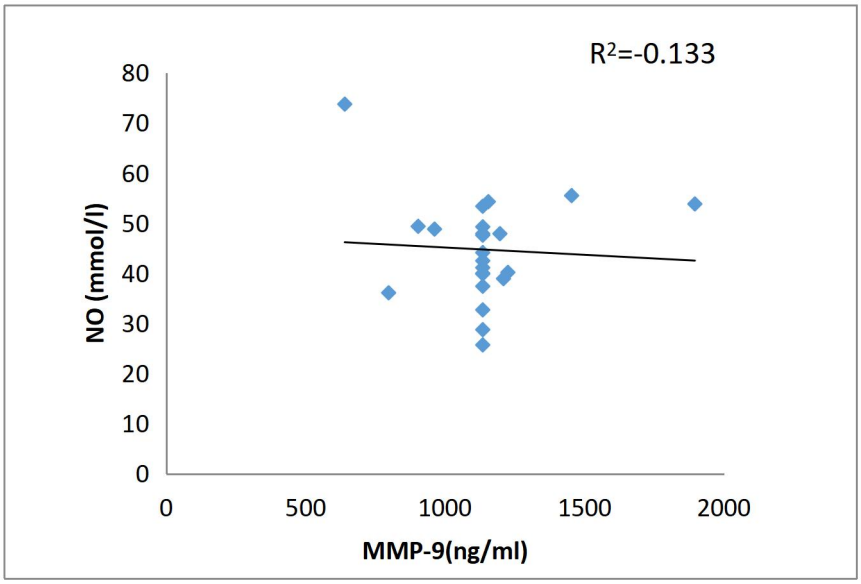


Fig.no-4.n : non significant negative correlation between and Matrix metalloproteinase -9 p> 0.05

o.Correlation between NO and TIMP-1

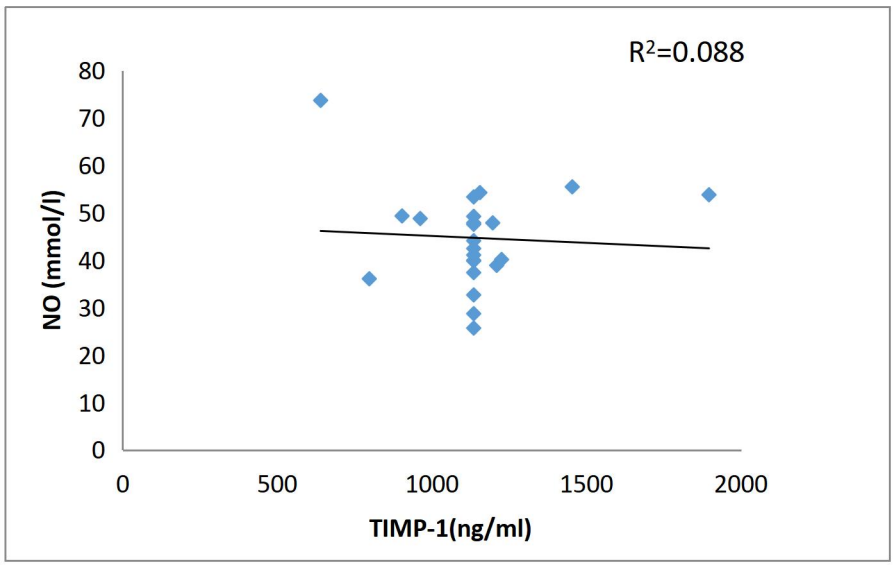


Fig.no-4.o : Non significant positive correlation between NO and Tissue inhibitors of metalloproteinase -1 p> 0.05

5.Correlation of Anthropometric and biochemical parameters with Matrix metalloproteinase -9 and Tissue inhibitors of metalloproteinase -1 in obese Hypertension group :

Correlation of Matrix metalloproteinase -9 and Tissue inhibitors of metalloproteinase -1 with Anthropometric parameters in Obese Hypertension

We found non-significant negative correlation between Body mass index(r=-0.184, p=0.331) and Matrix metalloproteinase -9 . There is a non significant negative correlation between Body mass index (r=-0.118,p=0.536) and Tissue inhibitors of metalloproteinase -1.

a .Correlation between BMI and MMP-9

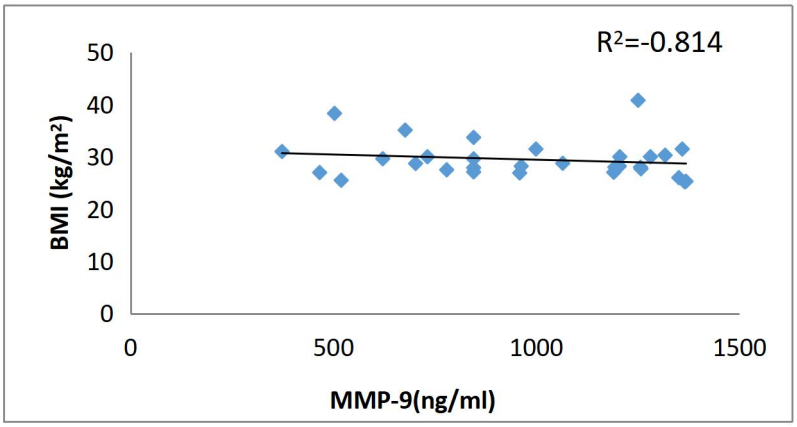


Fig.no-5.a: non-significant negative correlation between Body mass index and Matrix metalloproteinase -9 p> 0.05

b .Correlation between BMI and TIMP-1

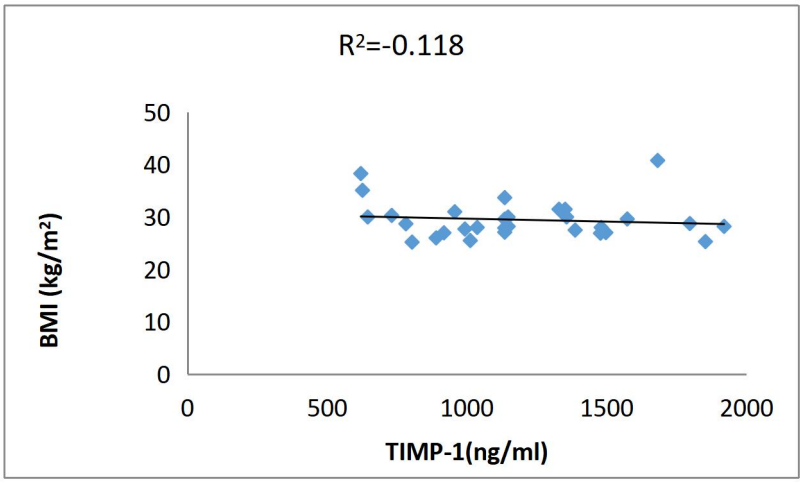


Fig.no-5.b : non significant negative correlation between Body mass index and Tissue inhibitors of metalloproteinase -1 with p> 0.05

Correlation of Matrix metalloproteinase -9 and Tissue inhibitors of metalloproteinase -1 with Physiological parameters in Obese Hypertension

We found non-significant positive correlation between Systolic blood pressure (r=0.072, p=0.706), Mean arterial presuure (r=0.028, p=0.882), and Matrix metalloproteinase -9 . There is a non significant negative correlation between Diastolic blood pressure (r=-0.055, p=0.773) and Matrix metalloproteinase -9 . Non significant negative correlation between Diastolic blood pressure (r=-0.128, p=0.500) Mean arterial presuure (r=-0.128, p=0.501) and Tissue inhibitors of metalloproteinase -1 and non significant positive correlation between Systolic blood pressure(r=0.000, p=0.998) and Tissue inhibitors of metalloproteinase -1IMP-1

c .Correlation between SBP and MMP-9

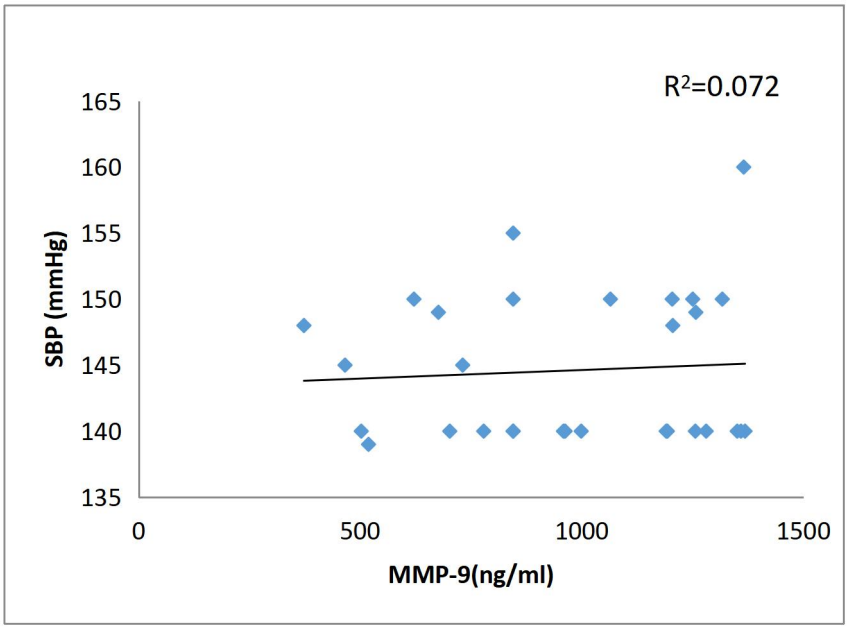


Fig.no-5.c: We found non-significant positive correlation between Systolic blood pressure (r=0.072, p=0.706), and Matrix metalloproteinase -9 with p> 0.05

d .Correlation between SBP and TIMP-1

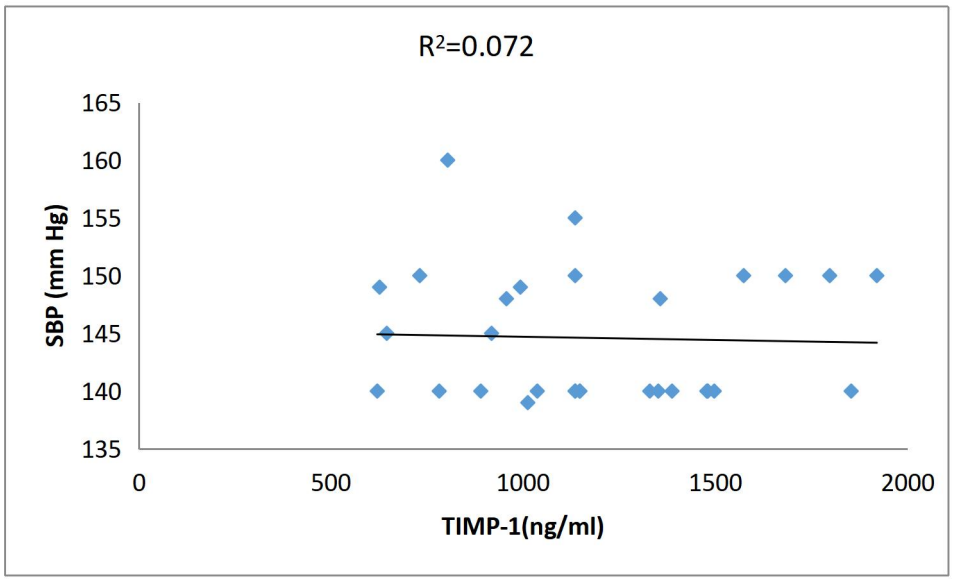


Fig.no-5.d : non significant positive correlation between Systolic blood pressure and Tissue inhibitors of metalloproteinase -1with p> 0.05

e .Correlation between DBP and TIMP-1

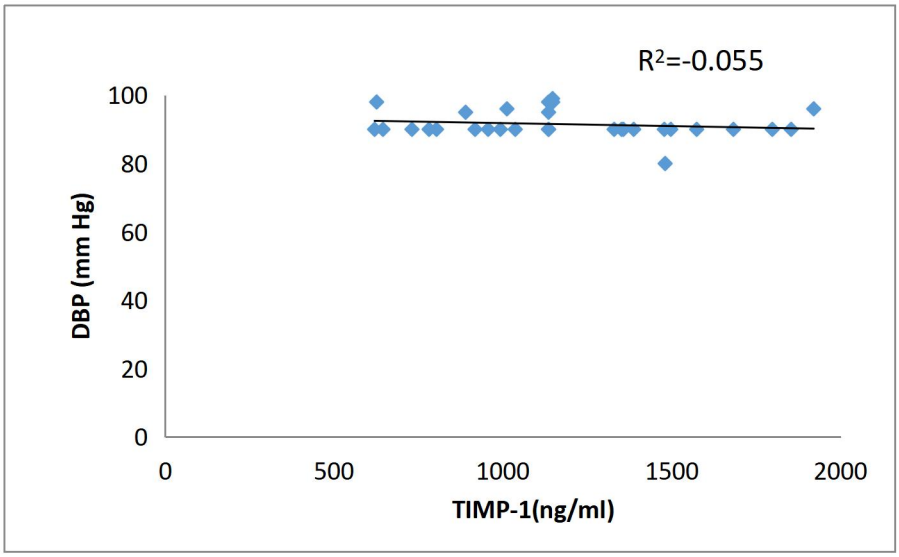


Fig.no-5.e: Non significant negative correlation between Diastolic blood pressure and Tissue inhibitors of metalloproteinase -1 with p> 0.05

f .Correlation between DBP andMMP-9

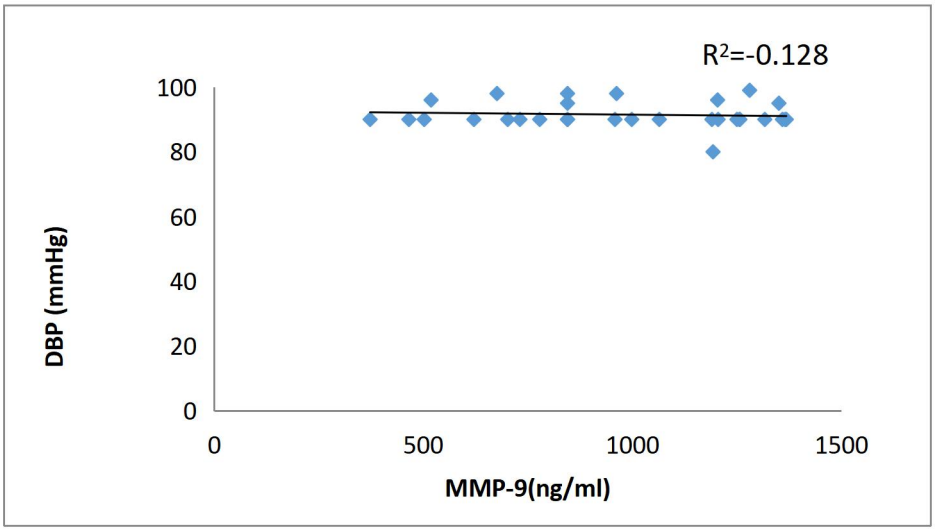


Fig.no-5.f : non significant negative correlation between Diastolic blood pressure and Matrix metalloproteinase -9 with p> 0.05

g.Correlation between MAP and MMP-9

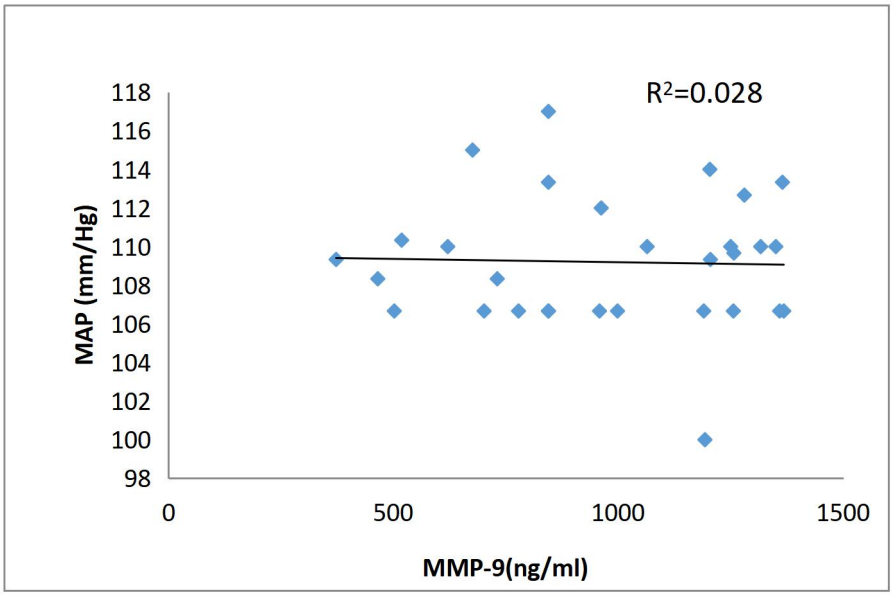


Fig.no-5.g: We found non-significant positive correlation between Mean arterial presuure and Matrix metalloproteinase -9 with p> 0.05

h. Correlation between MAP and TIMP-1

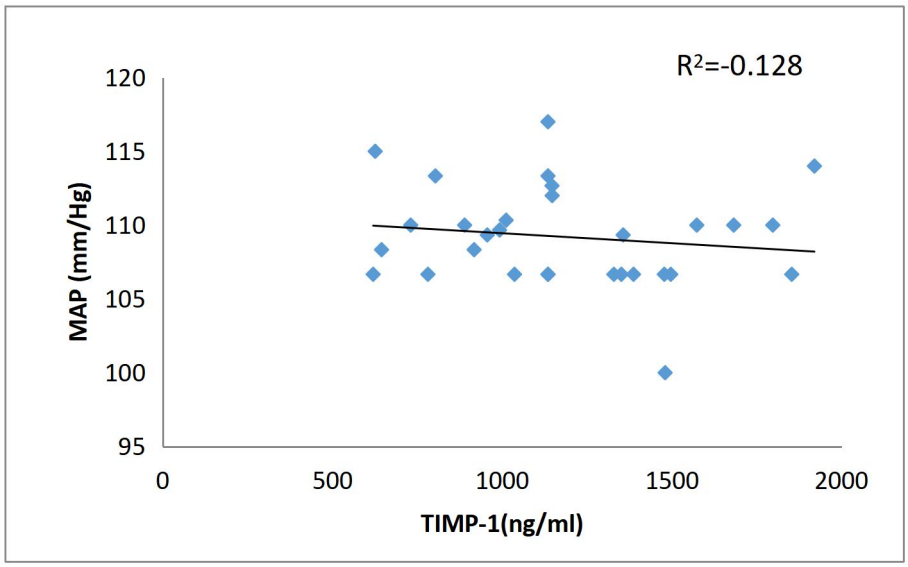


Fig.no-5.h : Non significant negative correlation between Mean arterial presuure and Tissue inhibitors of metalloproteinase -1 with p> 0.05

Correlation of Matrix metalloproteinase -9 with Tissue inhibitors of metalloproteinase -1 in Obese Hypertension

We found non-significant positive correlation between Matrix metalloproteinase -9 (r=0.299, p=0.109) and Tissue inhibitors of metalloproteinase -1 in obese **Hypertension** individuals.

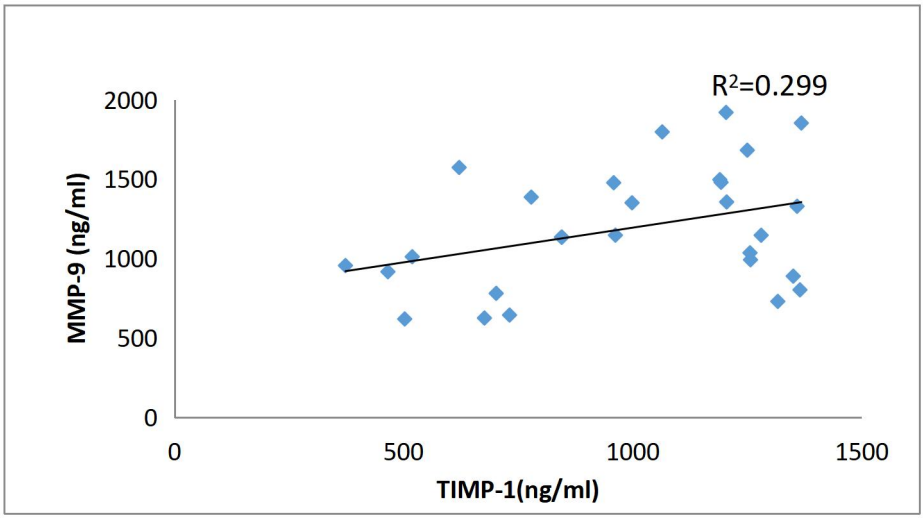


Fig.no-5.I: non-significant positive correlation between Matrix metalloproteinase -9 and Tissue inhibitors of metalloproteinase -1 with p> 0.05

Correlation of Matrix metalloproteinase -9 and Tissue inhibitors of metalloproteinase -1 with oxidative stress parameters in Obese Hypertension

We found non-significant negative correlation between Malondialdehyde (r= -0.168, p=0.374), significant negative correlation between NO (r=-0.580, p=0.001) and Matrix metalloproteinase -9 , non significant positive correlation between Reduced glutathione (r=0.151, p=0.427), Duration of Hypertension(r=0.085,p=0.653) ,Duration of treatment (r=0.086,p=0.664) and Matrix metalloproteinase -9 respectively.

There is non-significant positive correlation between Malondialdehyde (r=0.051, p=0.789), Reduced glutathione (r=0.051, p=0.789) non significant negative correlation between Nitric oxide (r=-0.138, p=0.468), Duration of Hypertension (r=-0.178, p=0.346) , Duration of treatment(r=0.232,p=0.235) and Tissue inhibitors of metalloproteinase -1 respectively.

j .Correlation between MDA and MMP-9

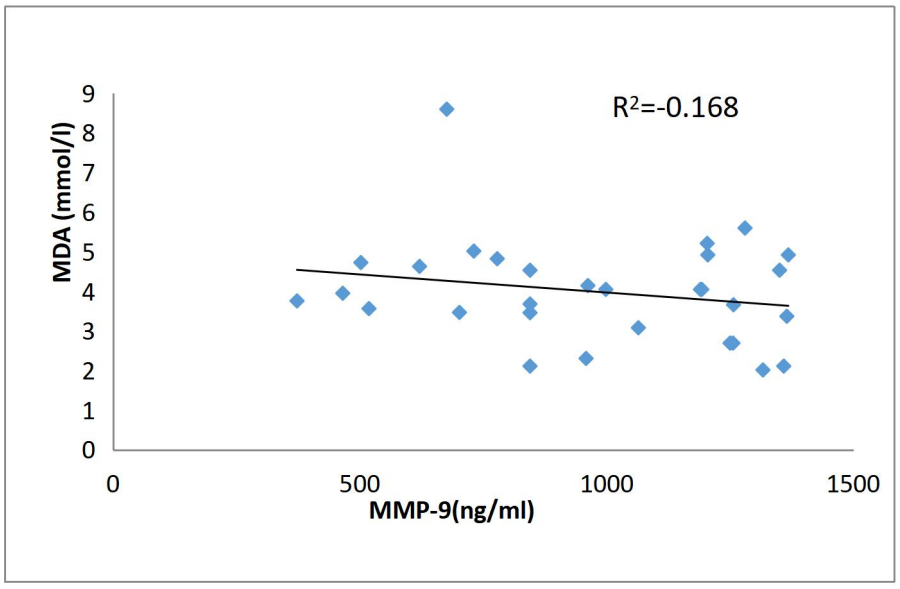


Fig.no-5.j : There is non-significant positive correlation between Malondialdehyde and Tissue inhibitors of metalloproteinase -1 with p> 0.05

k .Correlation between MDA and TIMP-1

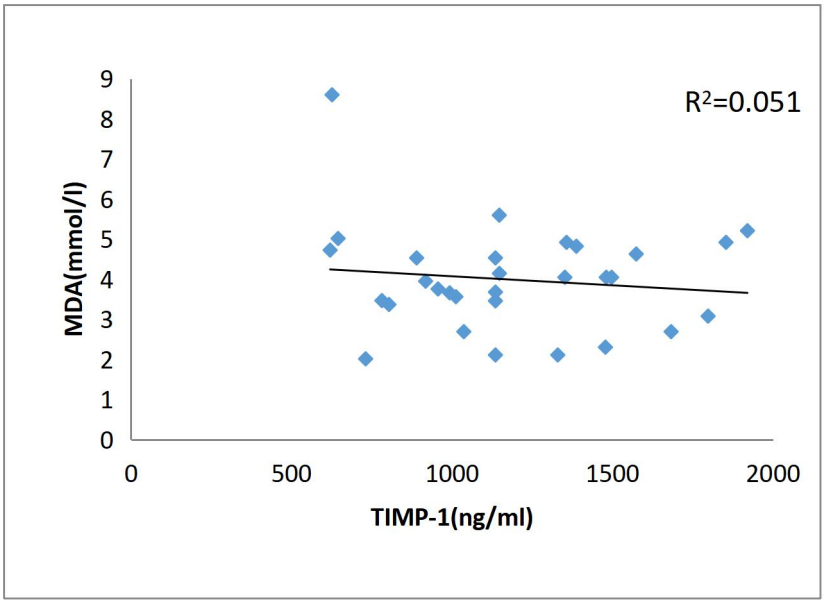


Fig.no-5.k : non-significant positive correlation between Malondialdehyde and Tissue inhibitors of metalloproteinase -1 with p> 0.05

l .Correlation between GSH and MMP-9

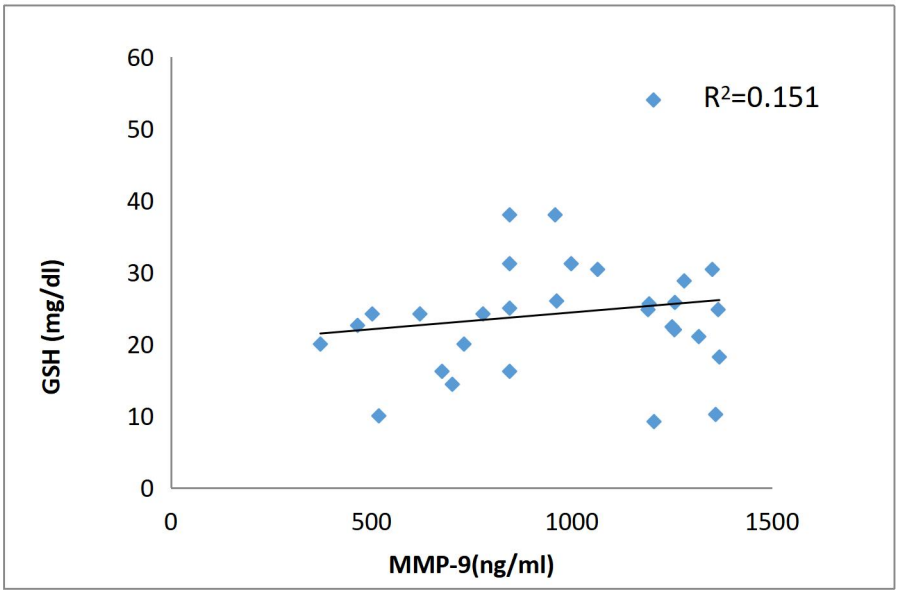
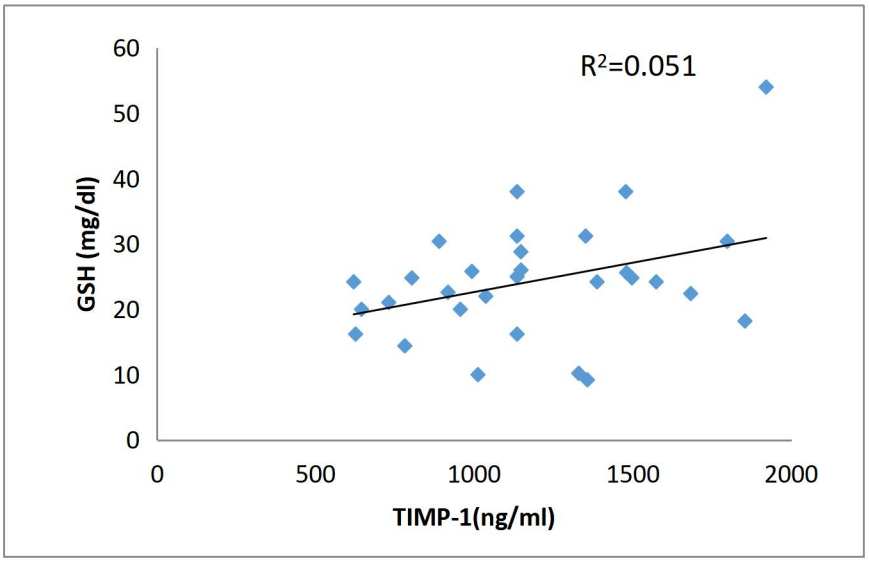


Fig.no-5.l: non significant positive correlation between Reduced glutathione and Matrix metalloproteinase -9 with p> 0.05

m .Correlation between GSH and TIMP-1



Fg.no -5.m : There is non-significant positive correlation between Reduced glutathione and TIMP-1 with p> 0.05

n.Correlation between NO and MMP-9

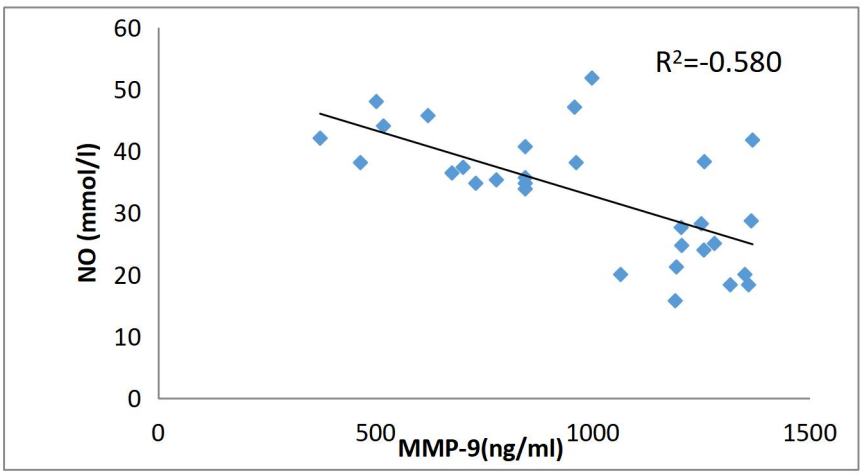


Fig.no-5.n: non significant negative correlation between Nitric oxide and Matrix metalloproteinase -9 with p> 0.05

o. Correlation between NO and TIMP-1

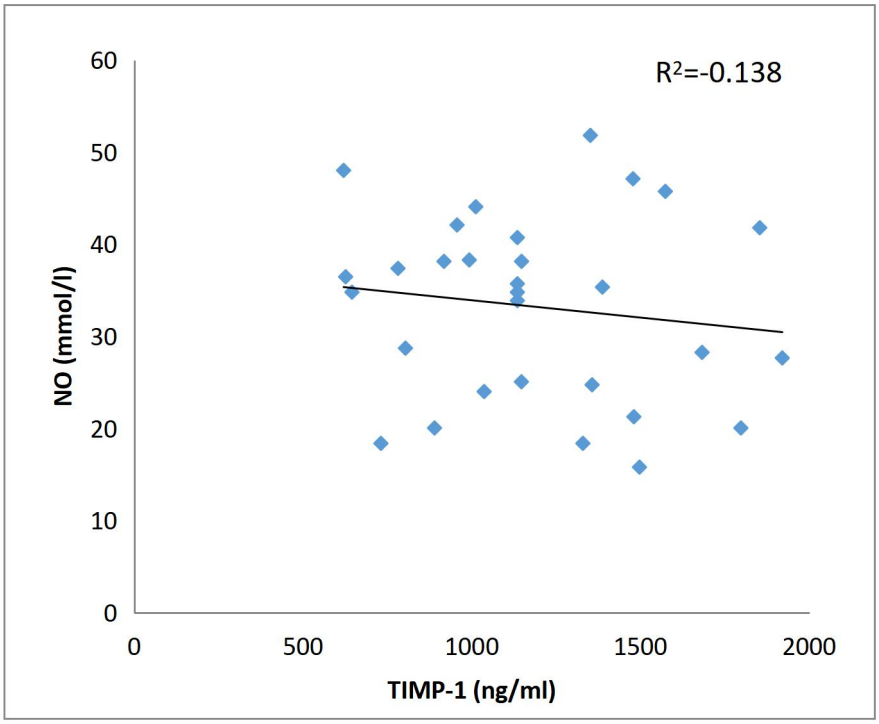


Fig.no-5.m: non significant negative correlation between Nitric oxide and Tissue inhibitors of metalloproteinase -1 with $p > 0.05$

DISCUSSION

In the present study, we have estimated the Blood pressure in all four groups, i.e., normal obese, hypertension obese, normal non-obese and hypertension non-obese, then estimated and compared MMP-9 and TIMP-1 among these groups. We found significant differences in MMP-9 levels (p - 0.020), a zinc-dependent protease responsible for the degradation of collagen, fibrinogen and vascular smooth muscle cells and causing vascular remodeling and dysfunction. TIMP-1, the endogenous inhibitor of metalloproteinase, show significant differences (p-0.032) in all four groups. Our results follow the A. Ritter et al. (September 2016) study of obesity-dependent effects on MMP-9 levels in treatment-resistant hypertension showed that MMP-9 levels are elevated in obese compared to non-obese individuals [35]. We found that the MMP-9 levels are higher in the obese HTN and obese control group than in the non-obese HTN and controls. Results on MMP-9 levels in obesity have been inconsistent. Several studies show lower levels of or no difference between obese and non-obese people.[36]

Our results demonstrated that there is no difference in TIMP-1 levels between obese and non-obese people. Whereas Soumaya Boumiza et al. (2021) demonstrated that the TIMP-1 levels are higher in obese than non-obese individuals [36]. In accordance with Flavia Mariana Valente et al. (2020) study [37], we found elevated levels of MMP-9 in the HTN group than Non-HTN groups. Hypertension is typically associated with vascular remodeling and rearrangement of several vascular wall constituents, including ECM. Many MMPs and tissue inhibitors of matrix metalloproteinase may have an impact on the vascular remodeling associated with hypertension. Elastin may degrade more quickly than collagen when MMP-9 activity is elevated, reducing the flexibility of vessel walls. High levels of MMP-9 have also been observed in acute cardiovascular events in individuals without a history of a specific clinical condition, suggesting that an increase in MMP-9 in healthy people may put them at risk for cardiovascular diseases. [37] We found increased levels of TIMP-1 in hypertensive than in non-hypertensive in both the obese and non-obese groups. Since TIMP-1 is an effective inhibitor of MMP-9, higher levels of TIMP-1 are likely linked

to the compensatory suppression of ECM breakdown with collagen deposition and vascular fibrosis [45] to increase vascular wall elasticity.

The membrane polyunsaturated fatty acids are damaged by oxidative stress, induced reactive oxygen species(ROS), which leads to the production of MDA. It has been shown that hypertensive individuals have elevated serum MDA levels (Manish Kumar Verma et al.,2019) [38]. In the present investigation, we found an elevation in MDA levels with increased MAP and increased levels are observed in both obese and non-obese individuals. The reduced activity of antioxidants like glutathione causes oxidative stress. To minimize the harmful effects of ROS, glutathione act as the first line of defense. The detoxification of super-oxide radicals by SOD produces hydrogen peroxide (H_2O_2). The enzymes catalase and glutathione peroxidase (GSPx) further reduced it to water. Glutathione per-oxidase uses GSH as a coenzyme to convert H_2O_2 into water. According to SG Patil's studies, antioxidant GSH and hypertension were demonstrated to be negatively correlated [39]. Our findings follow the previous results, which indicate decreased levels of GSH with increased MAP levels irrespective of obese and non-obese groups.Reactive oxygen species (ROS) are produced more often in hypertensive individuals and scavenge NO. Increased ROS generation and disturbed NO and ROS levels reduce NO bio-availability. This reduces endothelial-dependent vasodilation and causes hypertension. We observed decreased nitric oxide levels as increasing mean arterial pressure, and our findings follow Matthias Hermann et al. Study [40]

In accordance with Soumaya Boumiza et al. (in 2021), We found that with increased BMI, there are no changes in the levels of MMP-9 and TIMP-1 in control and HTN as in obese and non-obese groups. Many studies show there is a significant difference between obesity and MMP-9. They show increased levels of MMP-9 with increasing BMI.[41]The cardiovascular effects of hypertension have been related to a pro-inflammatory state, which may lead to increased synthesis of MMPs and TIMPs. The interaction between MMPs and their tissue inhibitors is crucial for controlling ECM. It has been determined that these structural and functional changes in the cardiac and vascular ECM cause their imbalance. Adipocytes are one of several cells involved in the production and regulation of MMP. Many authors hypothesized that reduced adiponectinemia and increased leptin

and pro-inflammatory cytokines levels frequently observed in obese people are responsible for obesity-associated MMP expression. MMP-9 is more excellent in obese with hypertension than in non-obese people.[42]

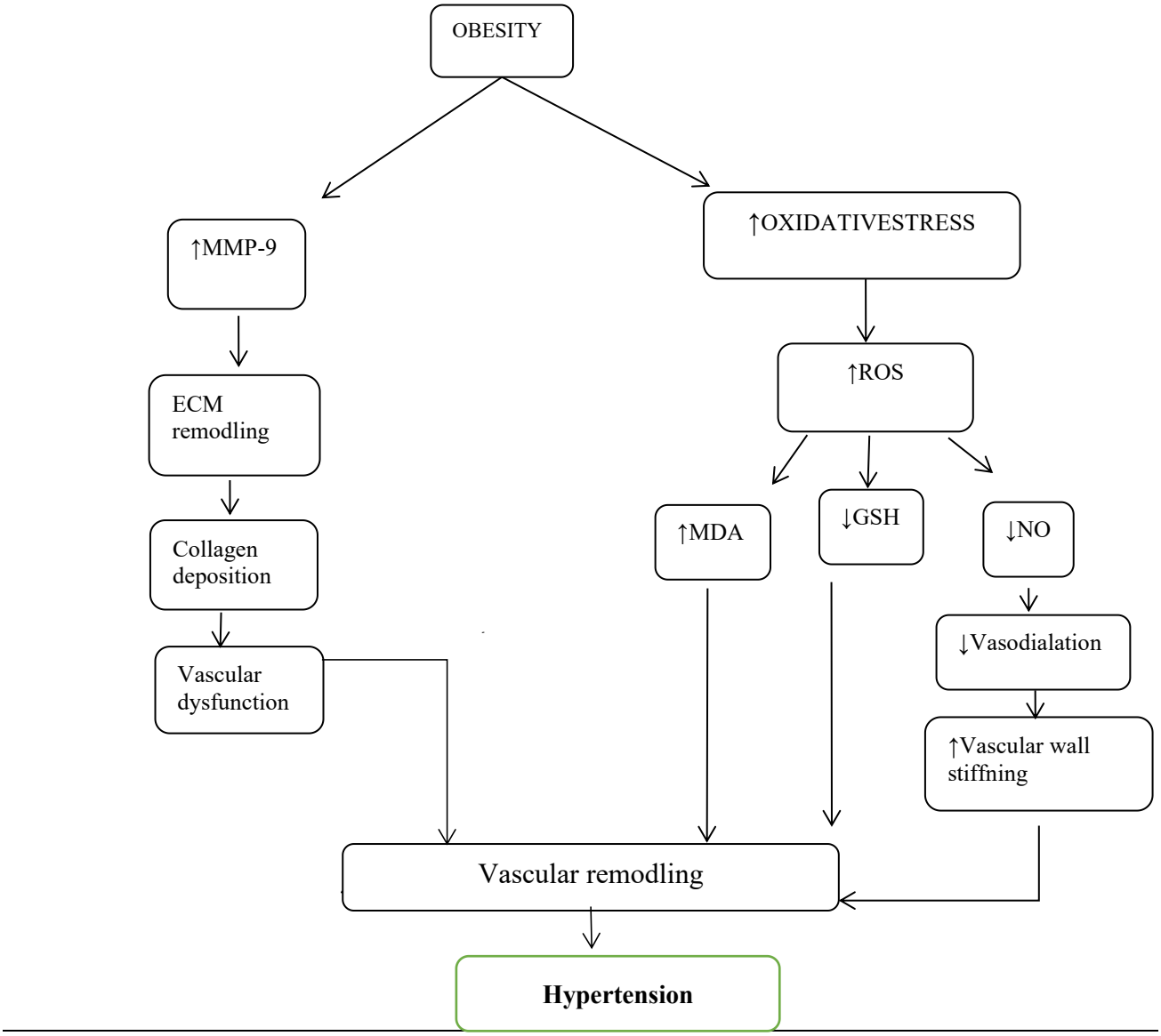
We observed the correlation of MMP-9 and TIMP-1 with increased blood pressure. The correlation between MMP-9 levels and hypertension was observed in various clinical and experimental research. Vascular remodeling and reorganization of several vascular wall constituents, including ECM, are frequently linked to hypertension. Several MMPs and tissue inhibitors of matrix metalloproteinase may mediate the vascular remodeling associated with hypertension. High MMP-9 activity may cause more elastin to break down than collagen, reducing elasticity.Nevertheless, suppose endogenous tissue inhibitor of matrix metalloproteinase-1 (TIMP-1) activity is reduced. In that case, this might result in a buildup of weakly cross-linked, immature, and unstable fibrin breakdown products, leading to incorrect collagen deposition.[43] we found a positive correlation between MMP-9 levels and SBP in all four groups, which is in accordance with Valente, F.M. et al. studies. This means that individuals with HTN may have greater levels of MMP-9 that lead to arterial wall stiffening and increased blood pressure.[43] But MMP-9 shows no changes with the elevated diastolic blood pressure or mean arterial pressure.

Usually, the MMP-9 activity is regulated by its tissue inhibitor TIMP-1. If the levels of TIMP decrease, the MMP-9 will be more active and causes vascular wall stiffening that leads to hypertension [44]. According to our findings, the TIMP-1 levels are increasing with increased MMP-9 in all four groups. Since TIMP-1 is a potent inhibitor of MMP-9, higher levels of TIMP-1 are likely linked to the compensatory suppression of ECM breakdown with collagen deposition and vascular fibrosis[45]. Our results are in accordance with Muzahir H et al. study. They found increased levels of MMP-9 and TIMP-1 in essential HTN patients than in control groups. [46]

As we observed in our study, oxidative stress is more in HTN, so the MDA levels are increased with elevated MAP and cause arterial wall stiffening. These

results are in agreement of previous of Manish Kumar Verma's study of Oxidative stress and biomarker of TNF-α, MDA and FRAP in hypertension. They found that MDA levels are more in hypertension than in control groups. [38] On the correlation of MDA with MMP-9 and TIMP-1 in hypertension-obese and non-obese groups, We found a non-significant negative correlation with MMP-9 and a significant (p=0.031) negative correlation in non-obese and non-significant negative correlation in the obese HTN group with TIMP-1. Whereas previous studies have shown that MMP-9 and MDA are positively correlated in arterial dysfunction [43], we did not find studies that can explain the correlation between MDA and TIMP-1. On comparison between the serum TIMP-1 levels with MDA in hypertensive and control subjects, we found a negative association between each other. As mentioned before, the GSH is decreased in the HTN groups compared to the control group. We found a negative correlation between MMP-9 and GSH in the HTN group and a positive correlation between TIMP-1 and GSH, which has not been evident in previous studies.because of the reduced levels of NO, vasodilation decrease, which would cause hypertension.[47] we found a negative correlation between the levels of MMP9 -NO and TIMP1-NO. Donald J Brown'S, Elements of the nitric oxide pathway can degrade TIMP-1 and increase gelatinase activity studies have shown that the TIMP and NO are positively correlated [48]. However, in contrast, our findings show a negative correlation between each other.

Possible mechanism by which MMP-9 and oxidative stress may cause Hypertension.



CONCLUSION AND SUMMURY:

From the above discussion, it can be concluded that elevated blood MMP-9 levels, Oxidative stress parameters and decreased antioxidant enzyme may cause vascular remodeling which may leads to hypertension. The TIMP-1 levels are rising along with the MMP-9 levels. Increased TIMP-1 levels are likely linked to the compensatory prevention of ECM deterioration. with collagen deposition and vascular fibrosis since TIMP-1 is a potent inhibitor of MMP-9. Moreover, we discovered a positive link between MMP-9 and MDA and a negative correlation between MMP-9 and GSH, NO. Our results also demonstrated the relationship between MMP-9 and oxidative stress while pointing to the possibility that MMP-9 and TIMP-1 may play a role in the pathogenesis of hypertension.

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MASTER CHRAT

OBESE CONTROL

Age	Sex	W/Hip	Wt/Ht	BMI	SBP	DBP	MAP	MMP-9	TIMP-1	MDA	GSH	NO
35	Male	0.91	0.55	27.15	120	80	93.3333	1083.67	1225.24	2.3052	54	40.16
49	Female	0.88	0.65	26.5	110	80	90	845.02	1135.12	2.7893	28.8	47.88
68	Female	0.93	0.68	37.5	110	90	96.6667	845.02	1135.12	2.6925	24.2	42.47
66	Female	0.95	0.75	30.5	120	90	100	1007.95	1196.65	2.8861	27.39	47.88
50	Female	0.83	0.6	26.9	110	90	96.6667	1490.69	1209.44	4.532	25.6	38.91
40	Male	0.91	0.55	27.15	110	90	96.6667	387.186	962.212	2.8861	18.4	48.79
47	Male	0.61	1.03	31.2	110	90	96.6667	845.02	1135.12	2.1116	30	47.58
36	Male	0.87	0.57	30.7	120	80	93.3333	845.02	1135.12	2.7893	34	39.98
43	Female	0.9	0.66	29.27	120	90	100	845.02	1135.12	1.6275	32	25.69
37	Male	0.96	0.55	27.3	120	80	93.3333	845.02	1135.12	1.4338	44	28.73
64	Female	0.91	0.71	27.4	110	70	83.3333	1278.2	1895.72	3.0798	36	53.81
43	Female	0.89	0.67	26	120	90	100	768.252	903.48	1.7243	18	49.34
42	Female	0.87	0.67	33.3	110	80	90	845.02	1135.12	2.2084	32	53.35
60	Female	0.87	0.68	29.5	110	80	90	420.18	640.38	1.2402	28	73.72
37	Female	0.85	0.56	27.2	110	90	96.6667	246.829	797.6	2.9829	23	36.09
36	Female	0.88	0.65	26.5	110	80	90	898.13	1155.17	1.7243	48	54.26
38	Female	0.89	0.67	26	110	80	90	293.69	1453.72	1.7243	32	55.48
45	Female	0.87	0.68	29.5	110	90	96.6667	845.02	1135.12	1.1434	24.8	32.68
56	Male	0.96	0.55	27.3	120	80	93.3333	845.02	1135.12	2.8861	17.6	44.11
42	Male	0.61	1.03	31.2	110	80	90	845.02	1135.12	2.3052	15.8	37.39
50	Male	0.61	1.03	31.2	110	70	83.3333	845.02	1135.12	2.402	28.6	49.25
58	Male	0.92	0.6	30	110	70	83.3333	845.02	1135.12	2.2084	26.8	41.1
56	Male	0.98	0.733	37	110	80	90	845.02	1135.12	1.8211	25.6	39.86

NON OBESE CONTROL

Age	Sex	W/Hip	Wt/Ht	BMI	SBP	DBP	MAP	MMP-9	TIMP-1	MDA	GSH	NO
48	Female	0.82	0.49	21.7	110	80	90	440	982.157	2.5957	18	26.11
38	Male	0.88	0.48	17.5	110	70	83.3333	570.532	579.34	2.6925	30.2	39.98
36	Female	0.86	0.55	21.9	110	80	90	732.477	519.68	2.9829	31	54.87
40	Female	0.9	0.54	18.6	110	70	83.3333	920.054	1361.52	3.0798	16	44.75
51	Female	0.81	0.5	23.12	120	80	93.3333	845.02	1135.12	2.2084	16	38.46
61	Female	0.87	0.59	18.35	120	80	93.3333	732.477	914.84	1.4338	16	39.58
50	Female	0.96	0.55	17.5	110	70	83.3333	648.85	585.04	2.6925	36.2	32.53
53	Male	0.87	0.59	18.35	110	90	96.6667	176.331	882.581	2.2084	28.3	42.44
51	Female	0.81	0.5	23.12	110	70	83.3333	227.347	901.847	2.4988	20.8	33.35
48	Male	0.94	0.53	22.3	120	80	93.3333	793.777	1115.76	2.3052	17	35.87
43	Male	0.91	0.5	20.7	110	70	83.3333	1272.63	841.96	1.7243	30.9	47.88
55	Female	0.81	0.4	19.6	110	70	83.3333	975.903	954.4	2.3052	24.2	40.34
58	Female	0.77	0.56	23	110	70	83.3333	221.382	507.02	2.402	26.4	35.11
52	Female	0.92	0.54	20	120	80	93.3333	945.303	937.6	2.5956	24.8	24.02
37	Female	0.93	0.17	24.4	120	80	93.3333	339.068	531.46	2.7893	17	43.47
48	Female	0.94	0.56	18.29	110	80	90	849.701	1149.24	2.6925	26.8	29.15
48	Male	0.94	0.53	22.3	110	80	90	845.02	1135.12	1.2402	24.6	57.61
38	Female	0.84	0.57	24.6	120	80	93.3333	845.02	1135.12	2.7893	16.8	37.49
55	Male	0.87	0.5	20	110	80	90	845.02	1135.12	2.5957	20.12	31.77
40	Female	0.75	0.45	22.7	110	80	90	845.02	1135.12	1.5307	32	29.82
43	Female	0.81	0.48	20.7	120	90	100	845.02	1135.12	2.9829	17	25.08
50	Female	0.89	0.51	18	120	80	93.3333	646.659	1008.4	1.0466	40	39.31
38	Female	1.04	0.68	23.9	120	70	86.6667	273.245	1042.52	1.9179	30	48.64
45	Male	0.81	0.48	20.7	120	80	93.3333	845.02	1135.12	2.3052	30	40.28
53	Female	1.04	0.68	23.9	120	70	86.6667	845.02	1135.12	2.0148	26.8	53.35
43	Female	0.9	0.54	18.6	110	80	90	845.02	1135.12	2.7893	16.2	35.87
45	Female	0.82	0.49	21.7	110	80	90	845.02	1135.12	2.5957	26.5	49.4
37	Female	0.75	0.45	22.7	120	90	100	845.02	1135.12	3.0798	23.5	55.48
36	Female	0.84	0.57	24.6	120	90	100	845.02	1135.12	1.6275	30.8	23.1
68	Male	0.87	0.5	20	110	80	90	845.02	1135.12	1.7243	26.46	44.11

OBESE HTN

Age	Sex	W/ Hip	Wt/Ht	BMI	SBP	DBP	MAP	MMP-9	TIMP-1	MDA	GSH	NO
Male	60	1.06	0.67	28.7	140	90	106	702	781.4	3.46	14.4	37.392
Female	40	0.82	0.6	26	140	95	110	1350.63	889.54	4.536	30.4	20.064
Male	55	0.95	0.06	25.3	140	90	106	1368.3	1853.28	4.919	18.2	41.8
Male	45	1.08	0.6	29.6	140	90	106	845	1135.11	2.1	16.2	33.896
Male	50	0.8	0.59	30.3	150	90	110	1316.93	730.74	2.01	21.04	18.392
Female	35	1.14	0.77	30	140	99	112	1280.58	1147.06	5.59	28.8	25.08
Female	54	0.91	0.74	35.1	149	98	115	676.33	626.14	8.59	16.2	36.48
Male	53	0.93	0.59	25.5	139	96	110	518.67	1011.86	3.56	10	44.08
Female	32	1.06	0.71	40.8	150	90	110	1250.3	1682.36	2.69	22.4	28.272
Female	55	0.87	0.52	38.3	140	90	106	502.26	620.14	4.72	24.2	48.032
Female	46	0.9	0.66	30	145	90	108	731.169	644.88	5.01	20	34.808
Male	52	0.88	0.52	27.7	149	90	109	1257.3	992.58	3.660	25.8	38.304
Male	62	1.07	0.7	28.7	150	90	110	1064.52	1797.48	3.07	30.4	20.064
Female	56	0.86	0.63	25.2	160	90	113	1365.3	803.42	3.37	24.8	28.728
Female	58	0.83	0.55	28	140	80	100	1193.03	1480.24	4.04	25.6	21.28
Female	52	0.54	0.54	28.2	150	96	114	1203.96	1920.1	5.20	54	27.664
Male	56	0.89	0.53	28	140	90	106	1256.2	1036.54	2.69	22	24.016
Female	65	0.91	0.59	28.2	140	98	112	962.3	1147.46	4.14	26	38.152
Male	60	1.047	0.66	29.6	150	90	110	621.156	1573.6	4.62	24.2	45.752
Male	43	1.03	0.69	31	148	90	109	372.89	956.3	3.75	20	42.104
Male	67	0.04	0.68	30	148	90	109	1205.2	1356.56	4.91	9.2	24.752
Male	65	1.09	0.7	26.9	145	90	108	465.606	917.54	3.95	22.6	38.152
Male	55	1.06	0.64	31.4	140	90	106	998.6	1351.34	4.047	31.2	51.832
Male	60	0.81	0.62	27.5	140	90	106	778.555 29	1387.14	4.82	24.2	35.36
Male	56	1.06	0.64	31.4	140	90	106	1359.03	1329.23	2.11	10.2	18.392
Female	59	0.77	0.57	26.9	140	90	106	958.56	1477.72	2.30	38	47.12
Male	38	1.06	0.65	27.1	150	95	113	845.02	1135.1	4.53	25	40.736
Female	38	0.89	0.63	27.0	140	90	106	1190.34	1496.98	4.047	24.8	15.808
Female	45	1.07	0.78	33.7	155	98	117	845.02	1135.11	3.680	38	35.72
Female	37	0.89	0.61	27.9	140	90	106	845.02	1135.11	3.46	31.2	34.808

NON-OBESE HTN

Age	Sex	W/Hip	Wt/Ht	BMI	SBP	DBP	MAP	MMP-9	TIMP-1	MDA	GSH	NO
65	Male	0.91	0.54	23.5	140	90	106	1155	1252.18	4.241	31	15.96
31	Female	0.85	0.56	20	150	90	110	1123.5	615	7.63	16	40.128
60	Female	0.88	0.52	20.25	145	90	108	1468.39	1607.44	3.3	28	24.624
47	Female	0.76	0.5	20.2	145	90	108	845.02	1135.11	4.14	18.4	28.424
48	Female	0.96	0.57	23.2	160	90	113	845.02	1135.11	4.3	27.2	53.2
67	Male	0.94	0.54	18.9	140	90	106	573.47	1032.56	4.53	41	48.64
56	Male	0.915	0.57	22.3	145	90	108	488.9	1452.84	3.37	28.2	37.088
50	Male	0.81	0.53	24.8	140	98	112	873.493	1474.06	3.37	16	37.088
62	Female	0.75	0.43	20	150	90	110	371.892	802.96	3.46	39.8	29.792
46	Male	0.94	0.54	19.1	145	98	113	718.1	1250.52	3.95	10	15.048
55	Female	0.96	0.57	23.6	150	90	110	1236.8	869.3	4.33	17	36.232
60	Female	0.91	0.68	21.9	145	94	111	463.105	937.08	4.8	47.2	22.952
64	Male	0.86	0.53	21.87	140	90	106	728.09	1792.18	4.0479	27.8	18.24
65	Female	0.91	0.59	19.44	145	99	114	819.986	963.66	3.85	28	38
69	Male	0.87	0.52	19.6	160	90	113	1070.09	1761.48	3.07	20.2	29.68
59	Male	0.94	0.46	17	140	90	106	1104.438	1671.1	2.9	24.8	25.992
70	Female	0.92	0.58	19.04	149	95	113	918.39	1356.5	4.91	38	20.8
52	Male	0.92	0.63	23.7	150	90	110	845	1135.11	3.46	26.8	57

B.L.D.E (DEEMED TO BE UNIVERSITY),

SHRI B.M. PATIL MEDICAL COLLEGE, HOSPITAL & RSEARCH CENTRE, VIJAYAPURA

Evaluation of MMP-9 and TIMP-1 in hypertension with or without obesity .A hospital based cross sectional study.

- Name ……
- Age: ……
- Sex: Male: ☐ Female: ☐
- Diet: Vegetarian ☐ Non-vegetarian ☐
- Lifestyle: active ☐ sedentary ☐
- Occupation:…….
- Obese ☐ Non-obese ☐
- Education: Educated ☐ UN-educated ☐
- Religion: Hindu ☐ Muslim ☐ Christian ☐ Others ☐
- Marital status: Married ☐ UN-married ☐

ANTRHROPOMETRIC PARAMETERS

- Height: …….
- Weight: …….
- Waist circumference. ……..
- Hip circumference: …….
- Waist-hip ratio: ……
- Waist-height ratio: …….
- BMI:…….

PHYSIOLOGICAL PARAMETERS

- Blood pressure:.....
- Hypertensive: Duration…….
- Had family history of HTN: YES☐ NO☐
- Cardiac heart disease: YES NO☐
- On Lipid-lowering drugs: YES ☐ NO☐ Duration:....
- On Anti-hypertensive drug.Drug name:…… YES ☐ NO☐ Duration:.....

BIOCHEMICAL PARAMETERS:

- MMP-9……..
- TIMP-1……..
- MDA…….
- GSH…….
- NO……

CONSENT FORM

B.L.D.E (DEEMED TO BE UNIVERSITY),

SHRI B.M. PATIL MEDICAL COLLEGE, HOSPITAL & RSEARCH CENTRE, VIJAYAPURA

RESEARCH INFORMED CONSENT FORM

Introduction: The study of "Evaluation of hypertension with or without obesity and its correlation with MMP-9 - a hospital based cross-sectional study." Will be conducted by Miss.Shahida H Byadagi MSc (Medical biochemistry) under the guidance of Dr. B B Devaranavadagi –HOD and professor department of Biochemistry, BLDE (Deemed to be University) Vijayapura You have been requested to participate in this research and participation is completely voluntary.

Purpose of the study: To study the levels of MMP-9 in obese and non-obese hypertension.

Procedure: the procedure is involved is, we are only collecting 4-5ml blood sample and
measuring your anthropometric parameters.

Risk and benefits: The only risk and possible discomfort you might get is while taking blood from your arm for the investigations. It may cause swelling, pain, redness (rarely happens) at the site from where the blood is drawn. You will also be benefitted by these investigations and you will be part of this study which is going to be useful to the community in future.

Privacy & Confidentiality: All the information regarding you, during the course of this study will be kept confidential. You will be identified in this research by the record code number.

Institutional/sponsor policy: As this study is non-invasive, there is no research related injury. There is no commitment to provide any compensation.

Financial incentives for participants: You will not receive any remuneration for participating in this study.

Voluntary Participation/Withdrawal: Your participation in the study is completely voluntary. If you decide not to participate in this study, it will not affect the quality of the medical care you receive at this institution.

Contact detail: If you have any question or queries, you may contact the study investigator Miss. Shahida H Byadagi (Mob. 7022439211 M.Sc. Medical biochemistry BLDE Vijayapura.) Dr. B B Devaranavadagi , HOD and Professor, Department of Biochemistry,
BLDE Medical College and Research Center,Vijayapur-58610 1, Mob.no-9448745957.

Authorization to publish result: The researcher may use information gathered from this study for presentation or publication but your identity will not be disclosed.

Consent statement: I am making a voluntary decision to allow myself to participate. My signature below indicates that I have read the information provided above, that I have been given the opportunity to ask question and the said question have been answered to my satisfaction.

Signature or left thumbprint of participants or legally authorized Representative

Participant's name:

- Signature/thumb print of participant:
- Name of the legally authorized Representative / guardian
- Signature of Representative /guardian
- Signature of co-guide/witness:
- Signature Researcher:
- Date:……

ಸಂಶೋಧನೆಗೆ ತಿಳಿಸಲಾದ ಸಮ್ಮತಿ ನಮೂನೆ

ಪರಿಚಯ: "ಸೂಕ್ಷ್ಮಕಾಯತೆಯೊಂದಿಗೆ ಅಥವಾ ಇಲ್ಲದೆಯೇ ಅಧಿಕ ರಕ್ತದೊತ್ತಡದ ಮೌಲ್ಯಮಾಪನ ಮತ್ತು MMP-9 ನೊಂದಿಗೆ ಅದರ ಪರಸ್ಪರ ಸಂಬಂಧ - ಇದು ಒಂದು ಆಸ್ಪತ್ರೆ ಆಧಾರಿತ ಅಡ್ಡ-ವಿಭಾಗದ ಅಧ್ಯಯನ." ಈ ಅಧ್ಯಯನವು ಡಾ. ಬಿ ಬಿ ದೇವರನವಾದಗಿ - ವೈದ್ಯಕೀಯ ಜೀವರಸಾಯನಶಾಸ್ತ್ರವಿಭಾಗದ ಮುಖ್ಯಸ್ಥಮತ್ತು ವಿಭಾಗದ ಪಾಠ್ಯಾಪಕರು, ಬಿಎಲ್ಡಿಇ (ಡಿಯು) ವಿಜಯಪುರ -ಅವರ ಮಾರ್ಗದರ್ಶನದಲ್ಲಿಮಿಸ್. ಶಾಹಿದಾ ಹೆಚ್ ಬಾಷಗಿ ಎಂಎಸ್ಸಿ(ವೈದ್ಯಕೀಯ ಜೀವರಸಾಯನಶಾಸ್ತ್ರ) ನಡೆಸಲಿದಾರ್ಥೆ.

ಈ ಸಂಶೋಧನೆಯಲ್ಲಿಭಾಗವಹಿಸಲು ನಿಮ್ಮನ್ನುವಿನಂತಿಸಲಾಗಿದೆ ಮತ್ತು ಭಾಗವಹಿಸುವಿಕೆಯು ಸಂಪೂರ್ಣವಾಗಿ ಸ್ವಯಂಪ್ರೇರಿತವಾಗಿದೆ.

ಅಧ್ಯಯನದ ಉದ್ದೇಶ: ಬೊಜ್ಜುಮತ್ತು ಸೂಕ್ಷ್ಮಕಾಯದ ಅಧಿಕ ರಕ್ತದೊತ್ತಡದಲ್ಲಿMMP-9 ಮಟ್ಟವನ್ನುಅಧ್ಯಯನ ಮಾಡಲು.

ಕಾರ್ಯವಿಧಾನ: ಕಾರ್ಯವಿಧಾನವು ಒಳಗೊಂಡಂತೆ , ನಾವು ನಿಮ್ಮ4-5ml ರಕ್ತದ ಮಾದರಿಯನ್ನುಮಾತ್ರಸಂಗ್ರಹಿಸುತಿದ್ದೇವೆ ಮತ್ತು ನಿಮ್ಮದೇಹದ ಅಳತೆಗಳು ಮತ್ತು ಅನುಪಾತಗಳ ನಿಯತಾಂಕಗಳನ್ನುಅಳೆಯುತಿದ್ದೇವೆ.

ಅಪಾಯ ಮತ್ತು ಪ್ರಯೋಜನಗಳು: ತನಿಖೆಗಾಗಿ ನಿಮ್ಮತೋಳಿನಿಂದ ರಕ್ತವನ್ನು ತೆಗೆದುಕೊಳ್ಳುವಾಗ ನೀವು ಪಡೆಯಬಹುದಾದ ಏಕೈಕ ಅಪಾಯ ಮತ್ತು ಸಂಭವನೀಯ ಅಸ್ವಸ್ಥತೆ ಎಂದರೆ ರಕ್ತವನ್ನುತೆಗೆದುಕೊಂಡ ಸ್ಥಳದಲ್ಲಿಊತ, ನೋವು, ಕೆಂಪು (ವಿರಳವಾಗಿ ಸಂಭವಿಸುತದ್ದೆ) ಕಾರಣವಾಗಬಹುದು. ಈ ತನಿಖೆಗಳಿಂದ ನೀವು ಸಹ ಪ್ರಯೋಜನ ಪಡೆಯುತ್ತೀರಿ ಮತ್ತು ಭವಿಷ್ಯದಲ್ಲಿಸಮುದಾಯಕ್ಕೆಉಪಯುಕ್ತವಾಗಲಿರುವ ಈ ಅಧ್ಯಯನದ ಭಾಗವಾಗುತ್ತೀರಿ.

ಗೌಪ್ಯತೆ- ಈ ಅಧ್ಯಯನದ ಅವಧಿಯಲ್ಲಿನಿಮಗೆ ಸಂಬಂಧಿಸಿದ ಎಲ್ಲಾಮಾಹಿತಿಯನ್ನು ಗೌಪ್ಯವಾಗಿ ಇರಿಸಲಾಗುತದ್ದೆ. ರೆಕಾರ್ಡ್ ಕೋಡ್ ಸಂಖ್ಯೆಯಿಂದ ಈ ಸಂಶೋಧನೆಯಲ್ಲಿ ನಿಮ್ಮನ್ನುಗುರುತಿಸಲಾಗುತದ್ದೆ.

ಸಾಂಸ್ಥಿಕ/ಪ್ರಾಯೋಜಕ ನೀತಿ: ಈ ಅಧ್ಯಯನವು ಆಕ್ರಮಣಕಾರಿಯಲ್ಲದ ಕಾರಣ, ಸಂಶೋಧನೆಗೆ ಸಂಬಂಧಿಸಿದ ಯಾವುದೇ ಗಾಯವಿಲ್ಲ.ಯಾವುದೇ ಪರಿಹಾರ ನೀಡುವ ಬದ್ಧತೆ ಇಲ್ಲ.

ಭಾಗವಹಿಸುವವರಿಗೆ ಆರ್ಥಿಕ ಪ್ರೋತ್ಸಾಹಗಳು: ಈ ಅಧ್ಯಯನದಲ್ಲಿಭಾಗವಹಿಸಲು ನೀವು ಯಾವುದೇ ಸಂಭಾವನೆಯನ್ನುಸ್ವೀಕರಿಸುವುದಿಲ್ಲ.

ಸ್ವಯಂಪ್ರೇರಿತ ಭಾಗವಹಿಸುವಿಕೆ/ಹಿಂತೆಗೆದುಕೊಳ್ಳುವಿಕೆ: ಅಧ್ಯಯನದಲ್ಲಿನಿಮ್ಮ ಭಾಗವಹಿಸುವಿಕೆಯು ಸಂಪೂರ್ಣವಾಗಿ ಸ್ವಯಂಪ್ರೇರಿತವಾಗಿದೆ. ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸದಿರಲು ನೀವು ನಿರ್ಧರಿಸಿದರೆ, ಈ ಸಂಸ್ಥೆಯಲ್ಲಿನೀವು ಪಡೆಯುವ ವೈದ್ಯಕೀಯ ಆರೈಕೆಯ ಗುಣಮಟ್ಟದ ಮೇಲೆ ಅದು ಪರಿಣಾಮ ಬೀರುವುದಿಲ್ಲ.

<u>ಸಂಪರ್ಕ ವಿವರ</u>: ನೀವು ಯಾವುದೇ ಪಶ್ನೆಗಳನ್ನು ಹೊಂದಿದರೆ, ನೀವು ಅಧ್ಯಯನ ತನಿಖಾಧಿಕಾರಿ ಮಿಸ್. ಶಾಹಿದಾ ಹೆಚ್ ಬಾಷಗಿ ಅವರನ್ನು ಸಂಪರ್ಕಿಸಬಹುದು (ಮೊ. 7022439211 M.Sc. ವೈದ್ಯಕೀಯ ಜೀವರಸಾಯನಶಾಸ್ತ್ರ ಡಾ. ಬಿ ಬಿ ದೇವರನವಾದಗಿ (ಮೊ.ಸಂ-9448745957) ಎಚ್ಒಡಿ ಮತ್ತು ಪ್ರಾಧ್ಯಾಪಕರು ಶ್ರೀ ಬಿ ಎಂ ಪಾಟೀಲ್ ವೈದ್ಯಕೀಯ ಕಾಲೇಜು ಮತ್ತು ಸಂಶೋಧನಾ ಕೇಂದ್ರ,ವಿಜಯಪುರ-58610 1.

<u>ಫಲಿತಾಂಶವನ್ನು ಪ್ರಕಟಿಸಲು ಅಧಿಕಾರ</u>: ಸಂಶೋಧಕರು ಈ ಅಧ್ಯಯನದಿಂದ ಸಂಗ್ರಹಿಸಿದ ಮಾಹಿತಿಯನ್ನು ಪ್ರಸ್ತುತಿ ಅಥವಾ ಪ್ರಕಟಣೆಗಾಗಿ ಬಳಸಬಹುದು ಆದರೆ ನಿಮ್ಮ ಗುರುತನ್ನು ಬಹಿರಂಗಪಡಿಸಲಾಗುವುದಿಲ್ಲ.

<u>ಸಮ್ಮತಿ ಹೇಳಿಕೆ</u>: ನಾನು ಭಾಗವಹಿಸಲು ಅವಕಾಶ ನೀಡಲು ಸ್ವಯಂಪ್ರೇರಿತ ನಿರ್ಧಾರವನ್ನು ಮಾಡುತಿದ್ದೇನೆ. ನಾನು ಮೇಲೆ ಒದಗಿಸಿದ ಮಾಹಿತಿಯನ್ನು ಓದಿದ್ದೇನೆ, ಪಶ್ನೆಯನ್ನು ಕೇಳಲು ನನಗೆ ಅವಕಾಶವನ್ನು ನೀಡಲಾಗಿದೆ ಮತ್ತು ಹೇಳಿದ ಪಶ್ನೆ ನನ್ನ ತೃಪಿಗ್ತೆ ಉತರ್ತಿಸಲಾಗಿದೆ ಎಂದು ಸೂಚಿಸುತದ್ದೆ.

- ಭಾಗವಹಿಸುವವರು ಅಥವಾ ಕಾನೂನುಬದ್ಧವಾಗಿ ಅಧಿಕೃತ ಪ್ರತಿನಿಧಿಯ ಸಹಿ ಅಥವಾ ಎಡ ಹೆಬೆರ್ಟಳು_____
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The Constituent College
SHRI B. M. PATIL MEDICAL COLLEGE, HOSPITAL & RESEARCH CENTRE, VIJAYAPURA

BLDE (DU)/IEC/ 719/2022-23 30/8/2022

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The Ethical Committee of this University met on **Friday, 26th August, 2022 at 3.30 p.m. in the Department of Pharmacology** scrutinizes the Synopsis of Post Graduate Student of BLDE (DU)'s Shri B.M.Patil Medical College Hospital & Research Centre from ethical clearance point of view. After scrutiny, the following original/ corrected and revised version synopsis of the thesis/ research projects has been accorded ethical clearance.

TITLE: "Evaluation of Hypertension with or without Obesity and its correlation with MMP-9 and TIMP-1 – A Hospital based Cross Sectional study".

NAME OF THE STUDENT/PRINCIPAL INVESTIGATOR: Miss. Shahida H Byadagi, MSc Medical Biochemistry.

NAME OF THE GUIDE: Dr. B. B. Devaranavadagi, Prof & HOD, Dept. of Biochemistry.

Dr. Santoshkumar Jeevangi
Chairperson
IEC, BLDE (DU),
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Chairman,
Institutional Ethical Committee,
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MEMBER SECRETARY
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Vijayapura-586103. Karnataka

Following documents were placed before Ethical Committee for Scrutinization:

- Copy of Synopsis/Research Projects
- Copy of inform consent form
- Any other relevant document

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PLAGIARISM VERIFICATION CERTIFICATE

1. **Name of the Student :** Miss. Shahida H. Byadagi **Reg. No. :** 19MSC001
2. **Title of the Dissertation :** "Evaluation of hypertension with or without obesity and its correlation with MMP9 and TIMP1- A Hospital Based Cross- Sectional Study ."
3. **Department :** Biochemistry
4. **Name of the Guide and Designation** : Dr. Basavaraj B. Devarnavadagi, HOD & Professor, Department of Biochemistry, BLDE (DU), Shri B. M. Patil Medical College, Hospital and Research Centre, Vijayapura, Karnataka.

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