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CHRONIC HYPERTENSION;
ASSESSMENT OF HAEMOSTATIC PARAMETER CHANGES

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ABSTRACT... Background: Hypertension is an important risk factor for cardiovascular morbidity and mortality. Myocardial infarction, and strokes, which are complications of hypertension, predominantly occur due to thrombosis of arterioles leading to ischemia and infarcts. NO suppression leads to hypertension associated with haemostatic changes that may endanger life. **Material and methods:** Rats were randomly divided into 2 groups equal in number, each contain 20 rats. Group (A) a control group given distillate water and Group (B) hypertension induced group receiving N^G-nitro-L-arginine methyl ester (L-NAME) for induction of hypertension. **Results:** Group 2 showed significant increases of mean blood pressure, plasma fibrinogen levels, significant reduction in mean values of percentages of platelets aggregation, significant increase in mean values of Plasminogen Activator Inhibitor-1 Antigen (PAI-1 antigen), significant increase in mean values of thromin-antithrombin complex (TAT)) and significant increase in mean values of soluble glycoprotein V (sGPV) . In contrast, platelets counts showed insignificant changes in its mean values. **Conclusion:** The present study demonstrates, the increase in plasma fibrinogen levels, fibrinolysis activities as indicated by increase in plasminogen Activator Inhibitor-1 Antigen (PAI-1 antigen), and systemic plasma thrombin which is detected by increases of mean plasma levels of thromin-antithrombin complex (TAT)) and soluble glycoprotein V (sGPV). Meanwhile decrease in nitric oxide in chronic hypertension produces slight decrease in platelets count and aggregation.

Key words: nitric oxide, hypertension, hemostatic parameters.

INTRODUCTION

Hypertension is an important risk factor for cardiovascular morbidity and mortality. Myocardial infarction, nephroangiosclerosis, and stroke which are complications of hypertension, predominantly occur due to thrombosis of arterioles leading to ischemia and infarcts[1]. The endothelium is not a static cell but a group of cells produces compounds that are important in regulating vascular homeostasis by elaborating factors such as angiotensin II, NO, endothelin, and prostaglandins[2]. The net effect is maintenance of normal vascular tone. So the normal endothelium maintains vascular health by providing a balance between vasodilatation and vasoconstriction. The endothelium also maintains normal blood viscosity, prevents abnormal blood clotting, and prevents abnormal bleeding in terms of a balance between plasminogen activator inhibitor-1 (PAI-1) and tissue plasminogen activator. It limits inflammation of the vasculature and it can suppress smooth muscle cell proliferation. These are functions of the normal endothelium. The opposite occurs in the presence of abnormal endothelium. Hypertension-particularly in high risk patients is a result of loss of this balance and the absence of the ability to vasodilate normally. Studies in humans suggest that hypertension is associated with a decrease in NO generation due to abnormal endothelium[3].

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An abnormal endothelium creates a phenotype that presents in patients with coronary artery disease, diabetics, and high-risk patients. Abnormally functioning endothelial cells cause decreased NO formation and a decrease in vasodilatation, as well as decreased angiotensin I and prostaglandin formation. The net effect is increased inflammation and hypertrophy of the smooth muscle cells. An abnormal endothelium promotes thrombosis and vasoconstriction and creates a situation ripe for establishment and rapid growth of atherosclerotic plaques.

Thrombotic risk associated with hypertension mainly targets the central nervous system, heart, and kidneys, leading to functional impairment and fibrosis of these tissues. It has been shown that one of the most important factors contributing thrombosis in hypertension is increase level of Plasminogen activator inhibitor-1 (PAI-1), a principal inhibitor of plasminogen activators which promotes thrombosis and fibrosis[4]. Feener et al reported that NO diminishes platelet-derived growth factor-stimulated PAI-1 expression in vascular smooth muscle increasing platelets aggregation via a cGMP-dependent pathway [5,6]. On the same time it has been shown that long-term inhibition of nitric oxide synthesis increases arterial thrombogenecity in rat carotid artery[7].

Chronic hypertension can lead to over expression of various genes such as anti-proteases which are tissue inhibitor of metalloproteinases (TIMP)[8] and plasminogen activator inhibitor-1 (PAI-1)[9] within the arterial wall[10]. The induction of the expression of tissue factor (TF) is the main initiator of thrombogenesis in the vascular wall[11]. As mentioned before essential hypertension is accompanied by chronic inhibition of NO synthesis. This can be initiated in animal through using with the L-arginine analogue N_-nitro-L arginine methyl ester (L-NAME)[12]. It produces hypertrophic remodeling in large arteries, whereas resistance arteries undergo inward eutrophic remodeling[13].

AIM OF STUDY

To study changes in hemostasis which accompany nitric oxide synthesis inhibition due to hypertension.

MATERIAL AND METHODS

1. ANIMAL USED

40 Male albino rats, weighing 200-250 gram were used. Rats were kept in metal free cages at temperature 22±1ºC and had free access to water.

2. EXPERIMENTAL DESIGN

Rats were randomly divided into 2 groups equal in number, each contain 20 rats.

Group (A) a control group:

Control rats were given distillate water

Group (B) hypertension induced group.

N^G-nitro-L-arginine methyl ester (L-NAME) ((Sigma) was added to the drinking water of the experimental group (B) in amounts 50 mg / Kg per day.

Both groups were fed with the same standard food for 8weeks. Mean blood pressure (MBP), measured by the tail-cuff method was recorded every 2 weeks. Every 2 weeks, blood was sampled. Blood was extracted from each animal by means of capillary glass tubing from the retro-orbital plexus by procedure described by Schremere[14]. Blood was centrifuged and plasma was frozen (-80ºC) for further analysis.

3. METHOD OF INDUCTION OF SEVERE HYPERTENSION[15]

To induce hypertension, rats received 50 mg/kg per day of N^G-nitro-L-arginine methyl ester (L-NAME) in drinking water.

4. METHOD OF MEASUREMENT OF BLOOD PRESSURE[16]

To verify hypertension development, mean blood pressure (MBP) was measured using the "tail cuff" method (Student Oscillograph, Harvard Rat Tail Blood Pressure Monitor, UK) in conscious rats. Each value was the average of three consecutive readings. Hypertensive rats (MBP higher than 130 mmHg) were used in the further experiments. Mean blood pressure (MBP) was measured by the tail-cuff method at beginning of experiment and every 2 weeks throughout the 8 weeks.

5. LABORATORY ANALYSIS

A. The Fibrinogen Level[17]

Fibrinogen level was measured according to the Clauses technique using an STA coagulation analyzer (Diagnostica Stago, Asnières, France.

B. Platelet Counts (N X10_5) /L[18]

Platelet counts were performed on whole blood collected on EDTA using a Bayer H1 counter.

C. Platelet Aggregation[19]

The percentages of platelet aggregation was performed in citrated platelet-rich plasma by the turbidimetric method using a 4-channel aggregometer (Coulter, Villepinte, France).Platelet aggregation was determined as the maximal change of light transmission after the addition of adenosine 5-diphosphate (ADP) (3 and 6 mol/L, Biodata, USA) or collagen (30 g/ml, Horm Nycomed, München, Germany).

(Were performed on whole blood collected on EDTA. was significantly decreased compared to controls (Fig. 2B) suggesting a desensitization of platelets in the L-NAME group)

D. Plasminogen Activator Inhibitor-1 Antigen (PAI-1 Antigen)[19,20]

Rat Plasminogen Activator Inhibitor-1 Antigen (PAI-1 antigen) was measured by ELISA techniques in a micro titer plate at 25˚C using a micro plate reader (Dynex Tech., USA) to monitor the changes in absorbance at 450 nm according to the manufacturer's directions

E. Thrombin– Antithrombin Complexes (TAT)[15,21]

Thrombin–antithrombin complexes (TAT) was measured using, a commercially available ELISA (Enzygnost TAT micro, Dade Behring, Paris-La Défense, France).

F-SOLUBLE GLYCOPROTEIN V (SGPV)[15,21]

Methods of measurement of soluble glycoprotein V (sGPV) for detection of plasma thrombin: Soluble glycoprotein V (sGPV) was measured using ELISA specific for rat sGPV developed by C.R.

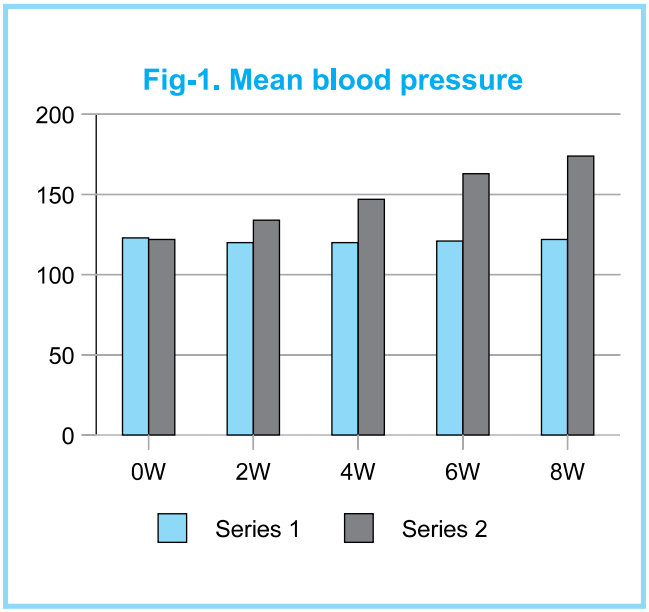
STATISTICAL ANALYSIS

All data are shown as mean ± SD. Statistical analysis of the results is performed by using student t test for quantitative data. $P < 0.05$ is considered statistically significant[22].

RESULTS

1. MEAN BLOOD PRESSURE, (FIG 1)

Mean values of mean blood pressure (MBP) in control group (group 1) were 123±8.1, 120±7.9, 120±8.1, 121±8.3, and 122±8.4 mmHg at weeks 0, 2, 4, 6 and 8 respectively.



While mean values of mean blood pressure (MBP) in hypertensive group (group 2) were 122±7.8, 134±8.1, 147±7.5, 163±7.9, and 174±8.7 mmHg in weeks 0, 2, 4, 6 and 8 respectively.

Comparing results of group 2 with group 1 there were significant increases of mean blood pressure at week 2,4,6 and 8 ($P < 0.05$).

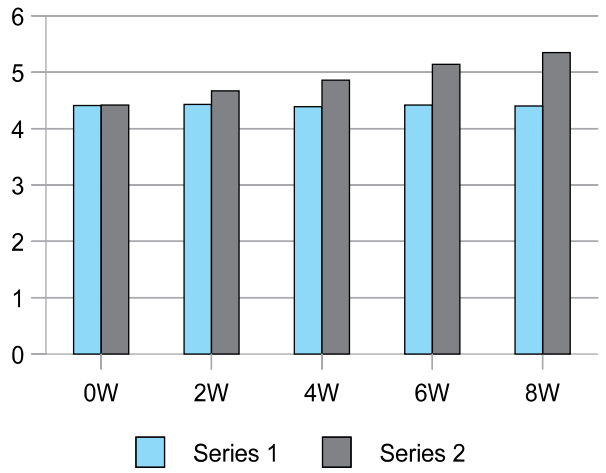
2. FIBRINOGEN LEVELS (FIG 2)

Mean values of plasma fibrinogen levels in control group (group1) were 4.41±0.21, 4.43±0.19, 4.39±0.18, 4.42±0.2, and 4.40±0.22 gram/litre at weeks 0, 2, 4, 6 and 8 respectively.

Mean values of plasma fibrinogen levels in hypertensive group (group2) were 4.42±0.23, 4.67±0.21, 4.86±0.26, 5.14±0.27, and 5.35±0.26 gram/litre at weeks 0, 2, 4, 6 and 8 respectively.

Comparing results of group 2 with group 1 there were significant increases of mean values of plasma fibrinogen levels at weeks 2,4,6 and 8 (P <0.05).

Fig-2. Fibrinogen levels



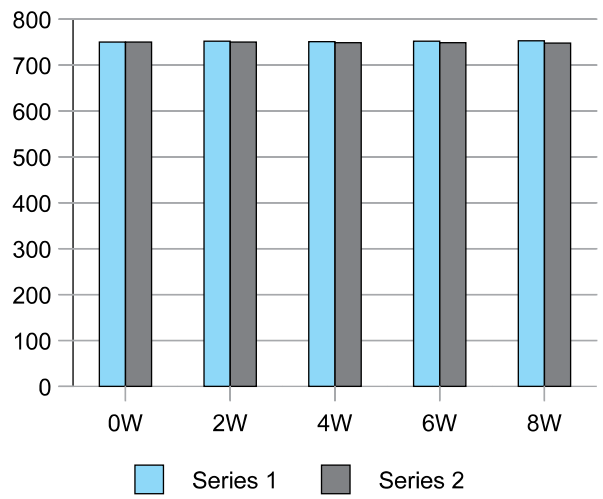
3. PLATELETS COUNTS (N X10$_5$) /L (FIG 3)

Mean values of platelets counts in control group (group1) were 750±22, 752±28, 751±30, 752±32 and 753±38 (X10^5) /L in weeks 0,2,4,6 and 8 respectively.

On the other hand Mean values of platelets counts in hypertensive group (group2) were 750±27, 750±23, 749±22, 749±19 and 748±31 (X10^5) /L at weeks 0,2,4,6 and 8 respectively.

Comparing results of group 2 with group 1 there were insignificant changes in mean values of platelets counts at weeks 0, 2,4,6 and 8 (P ≥0.05).

Fig-3. Platelets counts (n x 10^5) / L



4. PLATELETS AGGREGATIONS

A. Response to ADP (FIG 4A AND 4B)

The mean percentages of platelet aggregation in response to 3 mol/L ADP in control group (group1) were 45±2.3%, 44.5±2.1%, 45.1±2.6%, 45.3±2.5% and 44.9±2.6% at weeks 0,2,4,6,and 8 respectively. Also The mean percentages of platelet aggregation in response to 6 mol/L ADP in control group (group1) were 75.2±4.3%, 74.8±4.6%, 75.8±5.1%, 75.3±4.9% and 75.5.±5.2% at weeks 0,2,4,6,and 8 respectively.

Meanwhile the mean percentages of platelet aggregation in response to 3 mol/L ADP in hypertensive group (group2) were 44±2.3%, 30±2.1%, 21±1.5%, 16±0.9% and 12±0.9% at weeks 0,2,4,6, and 8 respectively and Also The mean percentages of platelets aggregation in response to 6 mol/L ADP in hypertensive group (group2) were 74.9±5.3%, 58.7.8±4.1%, 41.9±2.7%, 30.5±1.7% and 23.4±0.1% at weeks 0,2,4,6,and 8 respectively.

Comparing results of group 2 with group 1 there were significant reduction in mean values of percentages of platelets aggregation at weeks 2,4,6 and 8 (P <0.05).

Fig-4A. Platelets aggregation in response to 3 mol/L ADP

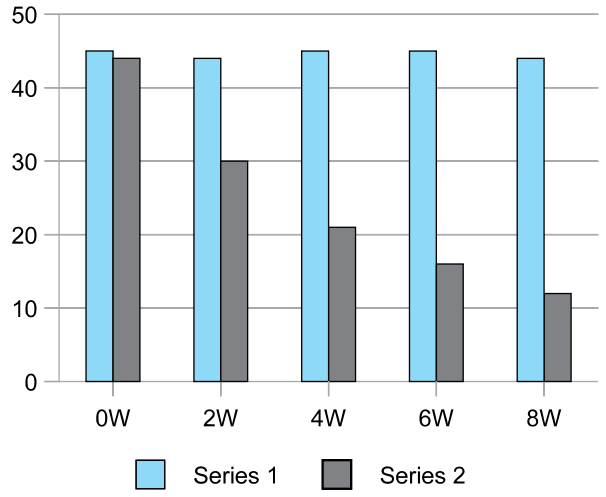
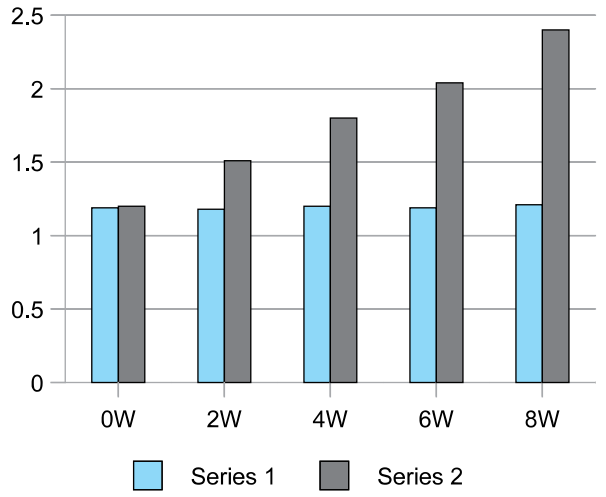


Fig-4B. Platelets aggregation in response to 6 mol/L ADP



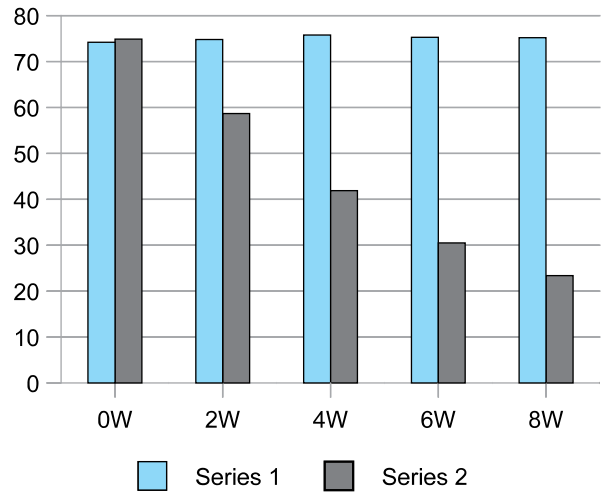
B. RESPONSE TO COLLAGEN (FIG 5)

The mean percentages of platelet aggregation in response to 30ug/mL collagen in control group (group1) were 46.1±2.9%, 45.8±2.7%, 46.2±2.9%, 45.8±2.8% and 45.9±3.1% at weeks 0, 2, 4, 6, and 8 respectively.

Also the mean percentages of platelet aggregation in response to 30ug/mL collagen in hypertensive group (group1) were 45.7±2.8%, 32.8±2.1%, 23.6±1.2%, 18.7±0.8% and 14±0.4% at weeks 0, 2, 4, 6, and 8 respectively.

Comparing results of group 2 with group 1 there were significant reduction in mean values of percentages of platelets aggregation in weeks 2,4,6 and 8 (P <0.05).

Fig-5. Platelets aggregation in response to collagen



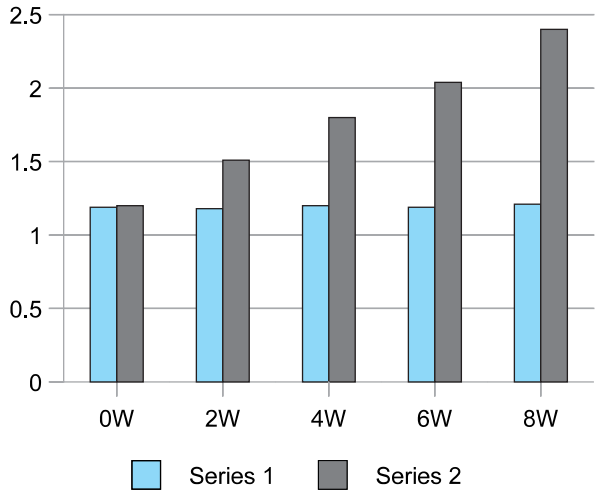
5. PLASMINOGEN ACTIVATOR INHIBITOR-1 ANTIGEN (PAI-1 ANTIGEN), (FIG 6)

Mean values of Plasminogen Activator Inhibitor-1 Antigen (PAI-1 antigen) in control group (group 1) were 1.19±0.058, 1.18±0.053, 1.2±0.071, 1.19±0.081 and 1.21±0.032 ng/ml at weeks 0, 2, 4, 6, and 8 respectively.

Mean values Plasminogen Activator Inhibitor-1 Antigen (PAI-1 antigen) in hypertensive group (group 2) were 1.20±0.057, 1.51±0.082, 1.8±0.083, 2.04±0.096 and 2.4±0.13 ng/ml at weeks 0, 2, 4, 6, and 8 respectively.

Comparing results of group 2 with group 1 there were significant increase in mean values of Plasminogen Activator Inhibitor-1 Antigen (PAI-1 antigen) at weeks 2,4,6 and 8 (P <0.05).

Fig-6. Plasminogen activator inhibitor-1 antigen



6. THROMIN-ANTITHROMBIN COMPLEX (TAT)), (FIG 7)

Mean values of thromin-antithrombin complex (TAT)) in control group (group 1) were 5.08±0.19, 4.98±0.14, 5.05±0.17, 5.08±0.21 and 4.96±0.19 ng/ml at weeks 0,2,4,6 and 8 respectively.

Meanwhile the mean values of thromin-antithrombin complex (TAT)) in hypertensive group (group 2) were 5.07±0.18, 7.21±0.21, 9.36±0.58, 13.52±0.98 and 16.56±1.015 ng/ml at weeks 0,2,4,6 and 8 respectively.

Comparing results of group 2 with group 1 there were significant increase in mean values of thromin-antithrombin complex (TAT) at weeks 2, 4, 6 and 8 (P <0.05).

7. SOLUBLE GLYCOPROTEIN V (SGPV),(FIG 8)

Mean values of soluble glycoprotein V (sGPV) in control group (group1) were 33.1±1.8, 32.9±1.7, 33.4±2.4, 33.3±2.6 and 32.9±2.2 ng/ml at weeks 0,2,4,6,and 8 respectively.

On the other hand mean values of soluble glycoprotein V (sGPV) in hypertensive group (group2) were 32.9±1.8, 39.3±2.3, 45.6±2.4, 53.7±2.9 and 61.3±2.9 ng/ml at weeks 0,2,4,6,and 8 respectively.

Comparing results of group 2 with group 1 there were significant increase in mean values of soluble glycoprotein V (sGPV) at weeks 2, 4, 6 and 8 (P <0.05).

Fig-7. Thromin-antithrombin complex.

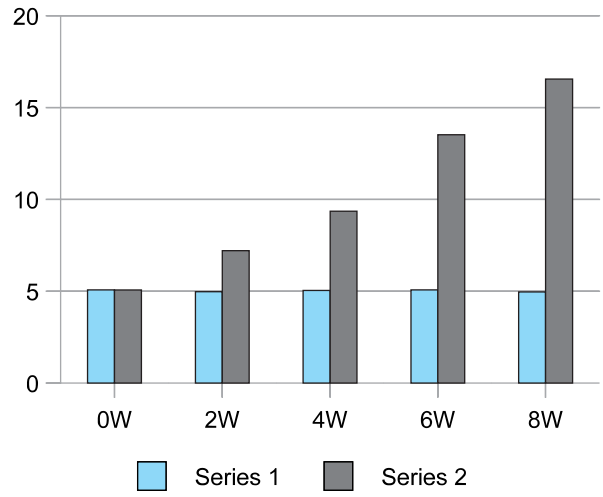
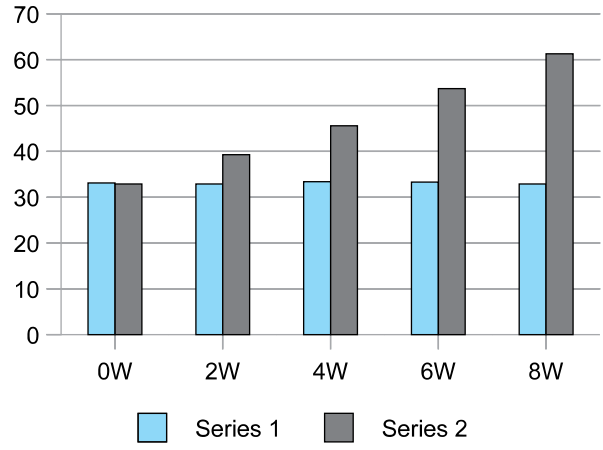


Fig-8. Soluble glycoprotein V.



DISCUSSION

Estimates of expenditures related to hypertension and its complications in 2007 indicate that total direct costs will

approach $49.3 billion. This expenditure's driving forces are medical durables, professional expenses, and hospital expenditures [23].

It is well known that Studies in humans suggest that hypertension is associated with a decrease in NO generation[24]. Thrombotic risk associated with hypertension mainly targets the central nervous system, heart and kidneys leading to functional impairment and fibrosis of these tissues[25,26]. It has been recently shown that an increase in intima/ media thickness of the carotid artery is correlated with the risk of brain infarction providing clinical evidence of a link between arterial wall morphologic remodeling and thrombotic risk[27].

The present study showed gradual significant increases of the main blood pressure of rats receiving N^G-nitro-L-arginine methyl ester (L-NAME) in drinking water throughout the experimental period. These results were in accordance with several studies using NO synthase inhibitor (L-NAME)[28-30]. The increased in mean blood pressure was accompanied with many hemostatic changes as shown in the present study.

The present study proved that there were significant increases in mean values of plasma fibrinogen levels in hypertensive group (group2) comparing with control group(1), as the levels were 4.42±0.23, 4.67±0.21, 4.86±0.26, 5.14±0.27, and 5.35±0.26 gram/litter at weeks 0, 2, 4, 6 and 8 respectively.

The increased in mean values of plasma fibrinogen levels in hypertensive group were in accordance with many studies searching hemostatic changes accompanying chronic hypertension. This finding underlines the important role of fibrinogen as a marker for assessing hypertension, and implies a possible contribution of nitric oxide in the pathophysiology of hypertension and cardiovascular disease.

Another study was done to detect role of nitric oxide deficiency accompanying hypertension on fibrinogen level using inducible nitric oxide synthase (INOS) inhibition (S-methylisothiourea). This study could prove that inhibition of nitric oxide metabolism could significantly decrease fibrinogen level in 12-week-old male stroke-prone spontaneously hypertensive rats[31].

Kubo-Inoue M and his coworkers could prove that long-term inhibition of endothelial NO synthesis due to chronic hypertension can increase arterial thrombogenecity due to increase of plasma fibrinogen level[15].

Vlachopoulos C et al (2007) and Fogari R et al (2008) could find positive correlation between levels of systolic blood pressure in hypertensive patients and serum fibrinogen level[32, 33].

On the other hand the present study showed Mean values of platelets counts in hypertensive group (group2), 750±27, 750±23, 749±22, 749±19 and 748±31 ($X10^5$) /L at weeks 0,2,4,6 and 8 respectively. Comparing results of group 2 with group 1 there were insignificant reduction in mean values of platelets counts at weeks 2,4,6 and 8 ($P > 0.05$).

These results were in accordance with results of Corseaux D et al (2002), who could find no difference in platelet count hypertensive model accompanying nitric oxide deficiency [34].

In contrast, Battinelli E and Loscalzo J (2000) could prove that exogenous and endogenous forms of nitric oxide (NO) can induce apoptosis in megakaryocytes[35].

Also Battinelli E et al (2001) got the same results. Their results demonstrate that (NO) facilitates platelet production, thereby establishing the essential role of NO in megakaryocyte development and thrombopoiesis[36].

These results can be explained by suggestion that a decrease in nitric oxide level in vessel wall is not accompanied by simultaneous decrease in platelets nitric oxide.

In relation to effects of chronic hypertension on platelets aggregations, the present study could prove significant reduction in mean values of percentages of platelets aggregation at weeks 2, 4, 6 and 8 in response to 3 and 6 mol/L ADP and also in response to 30ug/mL collagen.

Our results are consistent with previous studies addressing the effect of L-arginine and NOS inhibitors on platelet aggregation. They found that platelet aggregation in response to ADP (3 or 6 mol/L) or collagen (30g/ml) was significantly decreased compared to controls in rats exposed to L-NAME ex vivo[35-37].

Chronic in vivo activation of platelets in the L-NAME animals may account for platelets desensitization to in vitro stimulation by ADP or collagen. In vitro, L-NAME potentiates platelet aggregation and platelet recruitment induced by ADP and in vivo L-NAME promotes agonist-induced platelet accumulation in the pulmonary vasculature in rabbits[38,39].

Concerning finrinolysis activities, the present study showed significant increases in mean values of Plasminogen Activator Inhibitor-1 Antigen (PAI-1 antigen) in hypertensive group (group 2) 1.20±0.057, 1.51±0.082, 1.8±0.083, 2.04±0.096 and 2.4±0.13 ng/ml at weeks 0, 2, 4, 6, and 8 respectively.

Another studies could find that Long-term inhibition of nitric oxide synthesis increases arterial thrombogenecity in rat and Impaired fibrinolysis. It has been demonstrated that t-PA, which promotes fibrinolysis, and its inhibitor PAI-1 are increased in plasma of human hypertensive patients[40,41]. Some authors consider PAI-1 as an early marker of hypertension[42].

Our finding could play an important role in the relationship between the arterial phenotype and the hypercoagulable state associated with hypertension.

In relation to effect of chronic hypertension associated with nitric oxide deficiency on plasma thrombin level the present study could detect enhancement of systemic plasma thrombin as proved by significant increases of mean plasma levels of thromin-antithrombin complex (TAT)) and soluble glycoprotein V (sGPV).

The mean values of thromin-antithrombin complex (TAT) in hypertensive group (group 2) were 5.07±0.18, 7.21±0.21, 9.36±0.58, 13.52±0.98 and 16.56±1.015 ng/ml at weeks 0,2,4,6 and 8 respectively. At the same time the mean values of soluble glycoprotein V (sGPV) in hypertensive group (group2) were 32.9±1.8, 39.3±2.3, 45.6±2.4, 53.7±2.9 and 61.3±2.9 ng/ml at weeks 0,2,4,6,and 8 respectively.

These results were in accordance with results detected by Smith et al 2003[43] and Nancy J et al 2006[44]. They found chronic administration of L-NAME could produce hypertension accomying by significant increases of thromin-antithrombin complex (TAT) and soluble glycoprotein V (sGPV).

Ravanat C and his coworker proved that, the increase in plasma levels of sGPV icould be considered as a reliable marker of in vivo thrombin-induced platelet activation[45]. Therefore, the generation of thrombin probably plays a central role in platelet activation.

CONCLUSIONS

The present study demonstrates, the increase in plasma fibrinogen levels, fibrinolysis activities as indicated by increase in plasminogen Activator Inhibitor-1 Antigen (PAI-1 antigen), and systemic plasma thrombin which is detected by increases of mean plasma levels of thromin-antithrombin complex (TAT) and soluble glycoprotein V (sGPV). Meanwhile decrease in nitric oxide in chronic hypertension produces slight decrease in platelets count and aggregation.

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