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### RESEARCH ARTICLE

## Value of plasma pentraxin 3 (PTX3) levels in predicting asymptomatic preclinical hypertensive-related atherosclerotic vascular disease in Egyptian patients with primary hypertension

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#### Abstract:-

**Background:** Pentraxins are generally considered acute phase proteins. Pentraxin 3 (PTX3), a long pentraxin, has been identified as an inflammatory biomarker, and its blood levels increase rapidly and dramatically in inflammatory conditions. Elevated plasma PTX3 levels were reported in patients with high systolic and diastolic blood pressures, and PTX3 can be an early marker of arteriosclerotic vascular damage. Carotid artery intima-media thickness (cIMT) is a well-established surrogate marker for subclinical atherosclerosis. However, the association between biomarkers of systemic inflammation and atherosclerosis progression in carotid artery is not well established.

**Aim of Study:** Was to evaluate the value of plasma PTX3 level as a predictor for asymptomatic preclinical hypertensive-related atherosclerotic vascular disease.

**Subjects and Methods:** 75 patients with primary hypertension who had no history or manifestations suggesting atherosclerotic vascular disease and 15 healthy subjects (control group) were included. Full history taking and thorough medical examination were done. Patients' weight, height, BMI, and blood pressure were assessed. Laboratory investigations (urine analysis, UACR, CBC, ESR, CRP, liver enzymes, urea, creatinine, blood sugar levels, HbA1c, uric acid, LDL cholesterol, HDL cholesterol, triglyceride, and PTX3 levels), ECG, echocardiography, and carotid ultrasonography (for measuring cIMT) were performed for all patients.

**Results:** The mean age of patients was 54.7±9.3 years (range, 38-73 years). Asymptomatic preclinical atherosclerotic vascular disease, as reflected by cIMT > 0.1cm, was reported in 39 patients (52%). Hypertensive patients with LDL cholesterol ≥ 100mg/dL or with HDL cholesterol < 50mg/dL for males and < 40mg/dL for females had significantly higher plasma PTX3 levels. Higher degrees of UACR and albuminuria were associated with significantly elevated plasma PTX3 levels. Plasma PTX3 levels were significantly higher in hypertensive patients with preclinical atherosclerotic vascular disease,

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as reflected by cIMT > 0.1cm, compared to those without. Significant positive correlation was noted between plasma PTX3 levels and cIMT in hypertensive patients.

**Conclusion:** Elevated plasma PTX3 levels were correlated with cIMT, a marker for preclinical atherosclerosis, in Egyptian hypertensive patients with asymptomatic preclinical atherosclerotic vascular disease, regardless of hypertension stages.

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### Introduction:-

Pentraxins are family of multimeric proteins, play critical role in innate immunity, generally considered acute phase immunity proteins<sup>[1]</sup>, and their effects influence a variety of phenomena such as inflammation, angiogenesis, tumorigenesis, and cell adhesion<sup>[2]</sup>. Pentraxins include short and long pentraxins; they have different protein size, synthesized by different genes, and produced by different cell types in response to different stimuli<sup>[3]</sup>. C-reactive protein (CRP), a well known short pentraxin, is produced by hepatocytes and other cell types during inflammation, and its release is induced by pro-inflammatory cytokines, mainly interleukin-6 (IL-6). Serum amyloid P-component (SAP), another short pentraxin, is solely synthesized by hepatocytes<sup>[4]</sup>. Pentraxin 3 (PTX3), the first identified long pentraxin, has been identified as a new inflammatory biomarker in 1990s. It conserves the C-terminal domain of classical short pentraxin, but differs with presence of an unrelated long N-terminal domain<sup>[5]</sup>. PTX3 is a multifunctional protein with complex regulatory roles in inflammation, extracellular matrix organization, and remodeling<sup>[6]</sup>. It possesses multifaceted properties extending beyond the fields of immunity and inflammation to cardiovascular disease<sup>[7]</sup>. Unlike CRP, which is synthesized mainly in liver, PTX3 is produced at the site of inflammation by macrophages, dendritic cells, neutrophils, fibroblasts, endothelial cells, and smooth muscle cells (SMCs). Production of PTX3 is induced by interleukin-1 (IL-1), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), oxidized low-density lipoprotein (ox-LDL), and microbial moieties<sup>[8]</sup>. Synthesized PTX3 can also be stored in neutrophil granules that, upon stimulation, release PTX3 rapidly into circulation<sup>[8]</sup>. PTX3 blood levels are low (< 2ng/mL) in normal conditions but increase rapidly (peak at 6-8 hours) and dramatically in inflammatory conditions<sup>[5]</sup>.

Cardiovascular diseases (CVD) are a major cause of death; according to World Health Organization (WHO), an estimated 17 million people globally die of cardiovascular diseases yearly<sup>[9]</sup>. Risk factors for CVD include traditional risk factors (e.g., advanced age, hyperlipidemia, hypertension, diabetes mellitus, obesity, etc.) and non-traditional risk factors (e.g., inflammation, oxidative stress, vascular calcifications, etc.)<sup>[10]</sup>. Atherosclerosis is the focal expression of a systemic disease affecting medium and large arteries. Although cholesterol accumulation in intimal layer is the major pathologic feature of atherogenesis, the inflammatory state represents the major detrimental factor for atherosclerosis progression. The earliest stage of atherosclerotic process is characterized by infiltration of macrophages and T-lymphocytes, which are progressively activated during the course of atherosclerotic process<sup>[11]</sup>. PTX3 is produced by different cell types potentially involved in atherosclerosis, in particular endothelial cells, smooth muscle cells, and macrophages<sup>[9]</sup>. Thus, in addition to its established risk factors, including hypertension, dyslipidemia, and diabetes mellitus, PTX3 may play a role in onset and progression of CVD<sup>[10]</sup>. PTX3 is involved in the molecular mechanisms leading to vascular damage and its elevated plasma levels can be a significant predictor in elderly hypertensive patients<sup>[12]</sup>. The sclerotic arteries possess high levels of PTX3, mainly localized within the endothelial cells and macrophages. PTX3 plays a role in the regulation of innate resistance to inflammatory reactions, and its high plasma levels were found to be related with the severity of coronary atherosclerosis<sup>[13]</sup>. PTX3, by interacting with P-selectin, a cell-adhesion molecule involved in tethering and rolling of leukocytes and platelets on activated endothelial cells, attenuates leukocytes recruitment at the site of inflammation, and this indicates a protective role of PTX3 in atherosclerosis<sup>[14]</sup>. In contrast, PTX3 was reported to induce deleterious effects in pathogenesis of atherothrombosis through increasing tissue factor (TF) expression in mononuclear and endothelial cells; the increased level of TF, the main orchestrator of coagulation cascade, causes thrombus formation, a feature of atherosclerosis. Additionally, PTX3 might interfere with plaque stability through binding to fibroblast growth factor 2 (FGF2), which plays a role in activation and proliferation of smooth muscle cells<sup>[5]</sup>. Plasma PTX3 levels were significantly elevated in patients with arterial inflammation who underwent percutaneous coronary intervention (PCI), and systemic PTX3 levels before PCI were associated with larger plaque area and a higher risk of plaque rupture<sup>[9]</sup>. Elevated plasma PTX3 levels were reported in patients with vasculitis, acute myocardial infarction, unstable angina pectoris, heart failure, systemic inflammation or sepsis, and psoriasis<sup>[15]</sup>.

Endothelial dysfunction is a characteristic trait in many cardiovascular disorders including arterial hypertension. In hypertension, the impaired nitric oxide pathway and enhanced smooth muscle vasoconstriction represent the main mechanisms producing endothelial dysfunction<sup>[16]</sup>. Local and systemic inflammatory process is a key role in development of endothelial dysfunction, suggesting a role for acute phase proteins. On this regard, high plasma levels of the inflammatory molecule PTX3 might be associated with endothelial dysfunction<sup>[17]</sup>. Elevated plasma levels of PTX3 have been found in patients with high systolic and diastolic blood pressures and in elderly hypertensive patients<sup>[12]</sup>. Elevated concentration of PTX3 was found inside atherosclerotic plaques, which suggests that PTX3 can be an early marker of arteriosclerotic vascular damage<sup>[18]</sup>.

Plasma PTX3 levels are increased in patients with carotid stenosis<sup>[19]</sup>. Carotid artery intima-media thickness (cIMT) is a well established surrogate marker of subclinical atherosclerosis, and is increasingly recognized as a significant independent predictor of adverse cardiovascular outcomes<sup>[5]</sup>. Scientists are interested in the association between biomarkers of subclinical atherosclerosis, namely cIMT, which represents an early stage of atherosclerotic disease, but the association of biomarkers of systemic inflammation with atherosclerosis progression in the carotid artery is not well established<sup>[18]</sup>.

### **Aim of study:-**

This study aimed to assess the plasma PTX3 levels in Egyptian patients with primary hypertension as well as to evaluate the value of plasma PTX3 level as a predictor for asymptomatic preclinical hypertensive-related atherosclerotic vascular disease.

### **Subjects And Methods:-**

This study was conducted at Internal Medicine Department, Benha University Hospital, Egypt, during the period from May, 2019 to March, 2020, included 75 hypertensive patients in addition to 15 age- and sex-matched healthy subjects as a control group. The study protocol was approved by ethics committee of Benha University hospital, and written informed consents were obtained from all participants. Patients with primary hypertension, aged more than 30 years, males or females, non-smokers or ex-smokers (quitting smoking for at least 6 months), with or without hypercholesterolemia, who had no history or manifestations suggesting atherosclerotic vascular disease, were included in this study. Exclusion criteria included the following patients: patients with secondary hypertension; patients with clinically-manifested atherosclerotic vascular disease (angina, myocardial infarction, peripheral artery disease, or transient ischemic attack "TIA"), with history of receiving treatments for atherosclerotic vascular diseases, or with heart failure; patients with diabetes mellitus, malignancies, immuno-inflammatory disorders, or receiving corticosteroids or other immuno-modulatory drugs; patients with infectious diseases at presentations; obese patients; and smokers.

The patients were subjected to full history taking and thorough medical examination. Anthropometric parameters, namely height (meter) and weight (kilogram) were measured by standard methods, and body mass index (BMI) was calculated according to the formula:  $BMI = \text{weight (kg)}/\text{height (m)}^2$  ( $kg$ : kilogram;  $m$ : meter); patients with BMI of  $25\text{kg}/\text{m}^2$  or more (i.e., overweight and obese patients) were excluded. Blood pressure was measured in sitting position after at least 30 minutes of rest, using standard mercury sphygmomanometer device; the blood pressure was measured 3 times at 5 minutes intervals, and the average of the 3 measurements was considered as blood pressure<sup>[20]</sup>. Blood pressure was categorized, according to the new American College of Cardiology (ACC)/American Heart Association (AHA), November 13, 2017, as follows: normal blood pressure (systolic pressure "SP" < 120mmHg, diastolic pressure "DP" < 80mmHg), elevated blood pressure (SP of 120-129mmHg, and DP < 80mmHg), stage-1 hypertension (SP of 130-139mmHg, DP of 80-89mmHg), stage-2 hypertension (SP > 140mmHg, DP > 90mmHg), and hypertensive crisis (SP > 180mmHg, DP > 120mmHg).

Urine samples were obtained from all patients at morning, general urine analysis was done (to exclude any urinary tract infections), and urinary albumin creatinine ratio (UACR) were determined (using spot urine samples); the patients were categorized according to UACR into: patient with UACR < 30 $\mu\text{g}/\text{mg}$  (normal), patients with UACR of 30-299 $\mu\text{g}/\text{mg}$  (microalbuminuria), and patients with UACR  $\geq 300\mu\text{g}/\text{mg}$  (macroalbuminuria). Venous blood sample (10mL) was obtained under aseptic conditions from antecubital vein of each patient after an overnight fast for 12 hours; the blood samples were collected in tubes containing EDTA (ethylene-diamine-tetraacetic-acid), centrifuged, and the plasma and serum were obtained, aliquoted, and stored at  $-20^{\circ}\text{C}$  until biochemical parameters were assayed. Laboratory investigations done included complete blood count (CBC), erythrocyte sedimentation rate (ESR), CRP, serum levels of liver enzymes, urea, creatinine, fasting and random blood sugar, hemoglobin-A1c (HbA1c), uric

acid, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (DHL) cholesterol, and triglyceride. The patients were categorized according to LDL cholesterol level (those with LDL cholesterol < 100mg/dL, and those with LDL cholesterol  $\geq$  100mg/dL) and to HDL cholesterol level (those with HDL cholesterol < 40mg/dL for males and < 50mg/dL for females, and those with HDL cholesterol  $\geq$  40mg/dL for males and  $\geq$  50mg/dL for females) [15]. Plasma PTX3 levels were determined using a commercial enzyme-linked immunosorbent assay (ELISA) kit, according to the manufacturer's instructions (Quantikine Human PTX-3/TSG-14 Immunoassay, DPTX30; R&D Systems, Inc. Minn., USA), and the result was expressed in ng/mL [8].

Electrocardiogram, echocardiography, and ultrasonography of both carotid arteries (for measurement of cIMT, as a marker of subclinical atherosclerosis) were performed for the included patients. Carotid artery ultrasonography was done by Philips SD800 ultrasound scanner using a 7.5 MHz high-resolution linear transducer. The cIMT measurement was according to the ultrasound scanning protocol recommended in Mannheim Intima Media Thickness Consensus 2006. Three measures of carotid IMT were done, and the mean value of these 3 measures was used as a mean cIMT. Mean cIMT values less than 0.1cm were considered normal and values more than 0.1cm reflect thickening of intima layer and validated as a measure of risk for atherosclerotic vascular disease burden [21].

### Statistical Methods:-

Statistical Package of Social Science (SPSS) version 20 was used for statistical analyses (SPSS Statistics for Windows, version 20; Armonk, New York: IBM Corp., Released 2011). The data were expressed in range, mean, and standard deviation "SD" (for quantitative data) and in frequencies and percentages (for qualitative data). Chi square ( $X^2$ ), Student t-test (for comparison between 2 groups), and ANOVA test "F value" (for comparison between more than 2 groups) were used as tests for significance. Pearson correlation coefficient ('r') was used for correlation between plasma PTX3 levels and other variables ('r' ranges from -1 to 1, as follows: -1: perfect negative correlation; 0: no linear correlation; 1: perfect positive correlation). P value less than 0.05 ( $P < 0.05$ ) was considered statistically significant [22].

### Results:-

This study included 75 hypertensive patients, 44 males (58.6%) and 31 females (41.4%), with a mean age of  $54.7 \pm 9.3$  years (range, 38-73 years). Of the included patients, 35 (46.7%) had stage-1 hypertension, 40 (53.3%) had stage-2 hypertension, and none (0.0%) had hypertensive crisis. Asymptomatic preclinical atherosclerotic vascular disease, as reflected by cIMT > 0.1cm, was reported in 39 (52.0%) patients (15 patients "20.0%" had stage-1 hypertension, and 24 patients "32.0%" had stage-2 hypertension); the remaining 36 patients (48%) had no preclinical atherosclerotic vascular disease where their cIMT was less than 0.1cm (20 patients "26.7%" had stage-1 hypertension, and 16 patients "21.3%" had stage-2 hypertension). The patients' distribution, numbers, and percentages are revealed in table 1.

The mean PTX3 level  $\pm$  SD was  $1.31 \pm 0.73$ ng/mL in hypertensive patients aged less than 49 years, was  $2.09 \pm 0.47$ ng/mL in hypertensive patients aged 50-59 years, was  $2.97 \pm 0.79$ ng/mL in hypertensive patients aged 60-69 years, and was  $3.61 \pm 0.62$ ng/mL in hypertensive patients aged 70 years or more. It was noted that progressive advancing in ages of hypertensive patients was associated with significant progressive increase in plasma PTX3 levels ( $F = 6.87$ ;  $P < 0.01$ ) (Table 2). Male hypertensive patients had mean plasma PTX3 level  $\pm$  SD of  $1.74 \pm 0.76$ ng/mL and female hypertensive patients had mean plasma PTX3 level  $\pm$  SD of  $1.89 \pm 0.81$ ng/mL; no significant differences were reported regarding plasma PTX3 levels between males and females ( $t$  test = 0.73;  $P > 0.05$ ) (Table 2). Hypertensive patients with LDL cholesterol levels  $\geq$  100mg/dL had significantly higher plasma PTX3 levels ( $2.31 \pm 0.79$ ng/mL) compared to those with LDL cholesterol levels < 100mg/dL ( $1.48 \pm 0.41$ ng/mL) ( $t$  test = 7.36;  $P < 0.001$ ). Mean plasma PTX3 levels in patients with HDL cholesterol levels < 50mg/dL for males and < 40mg/dL for females ( $2.59 \pm 0.31$ ng/mL) were significantly higher compared to its levels in patients with HDL cholesterol levels of  $\geq$  50mg/dL males and  $\geq$  40mg/dL for females ( $1.09 \pm 0.21$ ng/mL) ( $t$  test = 4.16;  $P < 0.001$ ) (Table 2).

The mean PTX3 level  $\pm$  SD was  $1.08 \pm 0.37$ ng/mL in hypertensive patients with UACR < 30 $\mu$ g/mg (normal), was  $1.47 \pm 0.65$ ng/mL in hypertensive patients with UACR of 30-299 $\mu$ g/mg (microalbuminuria), and was  $2.81 \pm 0.71$ ng/mL in hypertensive patients with UACR  $\geq$  300 $\mu$ g/mg (macroalbuminuria) (Figure 1). Plasma PTX3 levels in patients with microalbuminuria were significantly higher compared to patients with normal urinary albumin ( $t$  test = 2.51;  $P < 0.05$ ). Plasma PTX3 levels were significantly higher in patients with macroalbuminuria compared

to patients with normal urinary albumin (t test = 6.97; P < 0.001). Plasma PTX3 levels were significantly higher in patients with macroalbuminuria compared to patients with microalbuminuria (t test = 5.92; P < 0.001) (Table 3).

Table 4 showed that plasma PTX3 level  $\pm$  SD in stage-1 hypertensive patients with cIMT < 0.1cm was  $1.39\pm 0.47$ ng/mL, in stage-2 hypertensive with cIMT < 0.1cm was  $1.51\pm 0.59$ ng/mL, in stage-1 hypertensive patients with cIMT > 0.1cm was  $3.13\pm 0.82$ ng/mL, and in stage-2 hypertensive patients with cIMT > 0.1cm was  $3.88\pm 1.01$ ng/mL. Plasma PTX3 levels were significantly higher in stage-1 hypertensive patients with preclinical atherosclerotic vascular disease, as reflected by cIMT > 0.1cm, compared to stage-1 hypertensive patients without preclinical atherosclerotic vascular disease as well as to controls ( $1.07\pm 0.57$ ng/mL). Also, plasma PTX3 levels were significantly higher in stage-2 hypertensive patients with preclinical atherosclerotic vascular disease compared to stage-2 hypertensive patients without preclinical atherosclerotic vascular disease as well as to controls (F = 50.37; P = 0.001) (Table 4; Figure 2).

Hypertensive patients with cIMT < 0.1cm had mean plasma PTX3 level  $\pm$  SD of  $1.43\pm 0.37$ ng/mL, and hypertensive patients with cIMT > 0.1cm had mean PTX3 level  $\pm$  SD of  $3.27\pm 0.61$  ng/mL. Hypertensive patients without preclinical atherosclerotic vascular disease, as reflected by cIMT < 0.1cm, had slightly higher plasma PTX3 levels ( $1.43\pm 0.37$ ng/mL) compared to controls ( $1.07 \pm 0.57$ ng/mL), whereas hypertensive patients with preclinical atherosclerotic vascular disease, as reflected by cIMT > 0.1cm, had significantly higher plasma PTX3 levels ( $3.27 \pm 0.61$ ng/mL) compared to those without preclinical atherosclerotic vascular disease ( $1.43\pm 0.37$ ng/mL) (t = 11.03; P < 0.001) and to controls (Table 4). Significant positive correlation was noted between plasma PTX3 levels and cIMT in hypertensive patients, where progressive increases in cIMT were associated with progressive increases in plasma PTX3 levels (r = 0.497; P = 0.001) (Figure 3).

**Table 1:-** Patients' distribution, numbers, and percentages regarding various parameters  
Number = 75 patients.

Item		Number (%)	
Hypertension	Stage-1 (n = 35)	cIMT > 0.1cm	15 (20.0%)
		cIMT < 0.1cm	20 (26.7%)
	Stage-2 (n = 40)	cIMT > 0.1cm	24 (32.0%)
		cIMT < 0.1cm	16 (21.3%)
cIMT	cIMT > 0.1cm (n = 39)	Stage-1	15 (20.0%)
		Stage-2	24 (32.0%)
	cIMT < 0.1cm (n = 36)	Stage-1	20 (26.7%)
		Stage-2	16 (21.3%)
Sex	Male (n = 44)	With cIMT > 0.1cm	25 (33.3%)
		With cIMT < 0.1cm	19 (25.3)
	Female (n = 31)	With cIMT > 0.1cm	14 (18.7%)
		With cIMT < 0.1cm	17 (22.7%)
Age (years)	< 49 years (n = 19)	With cIMT > 0.1cm	6 (8.0%)
		With cIMT < 0.1cm	13 (17.3%)
	50-59 years (n = 23)	With cIMT > 0.1cm	13 (17.3%)
		With cIMT < 0.1cm	10 (13.3%)
	60-69 years (n = 26)	With cIMT > 0.1cm	15 (20.0%)
		With cIMT < 0.1cm	11 (14.7%)
	$\geq$ 70 years (n = 7)	With cIMT > 0.1cm	5 (6.7%)
		With cIMT < 0.1cm	2 (2.7%)
UACR ( $\mu$ g/mg)	< 30 $\mu$ g/mg (n = 23)	With cIMT > 0.1cm	7 (9.4%)
		With cIMT < 0.1cm	16 (21.3%)
	30-299 $\mu$ g/mg (n = 31)	With cIMT > 0.1cm	15 (20.0%)
		With cIMT < 0.1cm	16 (21.3%)
	$\geq$ 300 $\mu$ g/mg (n = 21)	With cIMT > 0.1cm	17 (22.7%)
		With cIMT < 0.1cm	4 (5.3%)
LDL (mg/dL)	< 100mg/dL (n = 42)	With cIMT > 0.1cm	13 (17.3%)
		With cIMT < 0.1cm	29 (38.7%)
	$\geq$ 100mg/dL	With cIMT > 0.1cm	26 (34.7%)

	(n = 33)	With cIMT < 0.1cm	7 (9.3%)
HDL (mg/dL)	< 50mg/dL (males); < 40mg/dl (females) (n = 35)	With cIMT > 0.1cm	27 (36.0%)
		With cIMT < 0.1cm	8 (10.7%)
	≥ 50mg/dL (males); ≥ 40mg/dL (females) (n =40)	With cIMT > 0.1cm	12 (16.0%)
		With cIMT < 0.1cm	28 (37.3%)

**Table 2:-** Correlation between plasma PTX3 levels and some parameters.

Item		PTX3 (ng/mL)	Statistical test	P value
Age (years)	< 49 years	1.31±0.73	F = 6.87	P < 0.01
	50-59 years	2.09±0.47		
	60-69 years	2.97±0.79		
	≥ 70 years	3.61±0.62		
Sex	Males	1.74±0.76	X <sup>2</sup> = 0.73	P > 0.05
	Females	1.89±0.81		
LDL (mg/dL)	< 100mg/dL	1.48±0.41	T = 7.36	P < 0.001
	≥ 100mg/dL	2.31±0.79		
HDL (mg/dL)	< 50mg/dL (for males); < 40mg/dl (for females)	2.59±0.31	T = 4.16	P < 0.001
	≥ 50mg/dL (for males); ≥ 40mg/dL (for females)	1.09±0.21		

**Table 3:-** Correlation between plasma PTX3 levels and UACR (albuminuria).

Item		PTX3 (ng/mL)	*P1	#P2	§P3
UACR	< 30µg/mg (normal)	1.08 ± 0.37	T = 2.51 P < 0.05	T = 6.97 P < 0.001	T = 5.92 P < 0.001
	30-299µg/mg (microalbuminuria)	1.47 ± 0.65			
	≥ 300µg/mg (macroalbuminuria)	2.81 ± 0.71			

\*P1: UACR &lt; 30µg/mg (normal) vs. UACR of 30-299 µg/mg (microalbuminuria)

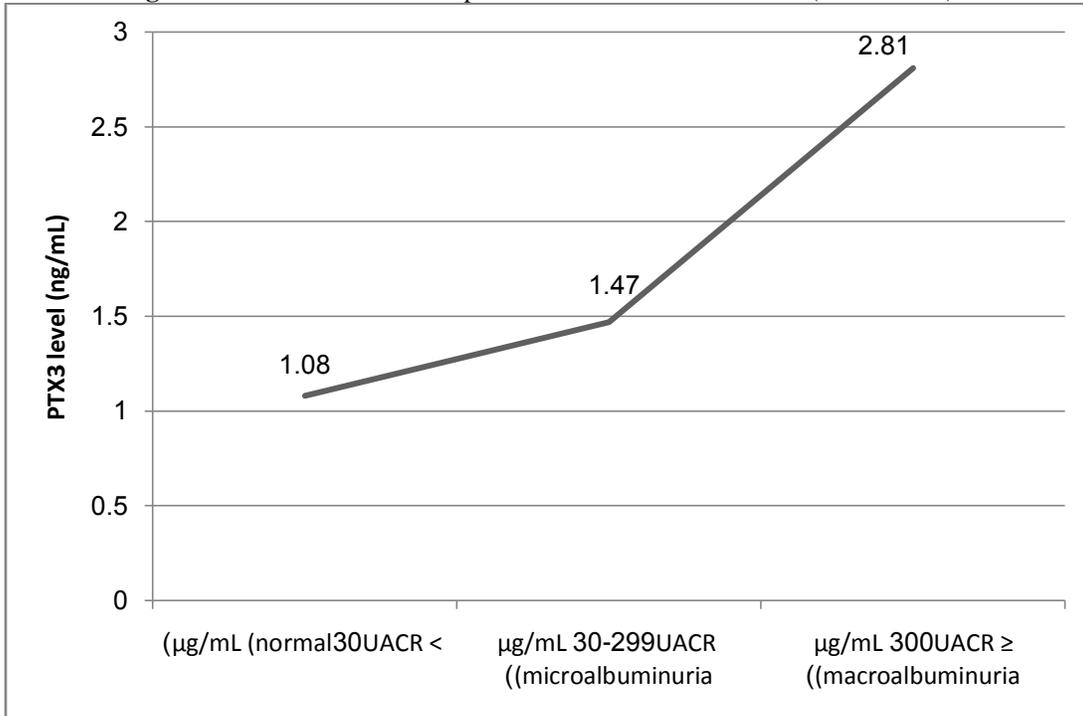
#P2: UACR &lt; 30µg/mg (normal) vs. UACR ≥ 300µg/mg (macroalbuminuria)

§P3: UACR 30-299µg/mg (microalbuminuria) vs. UACR ≥ 300µg/mg (macroalbuminuria)

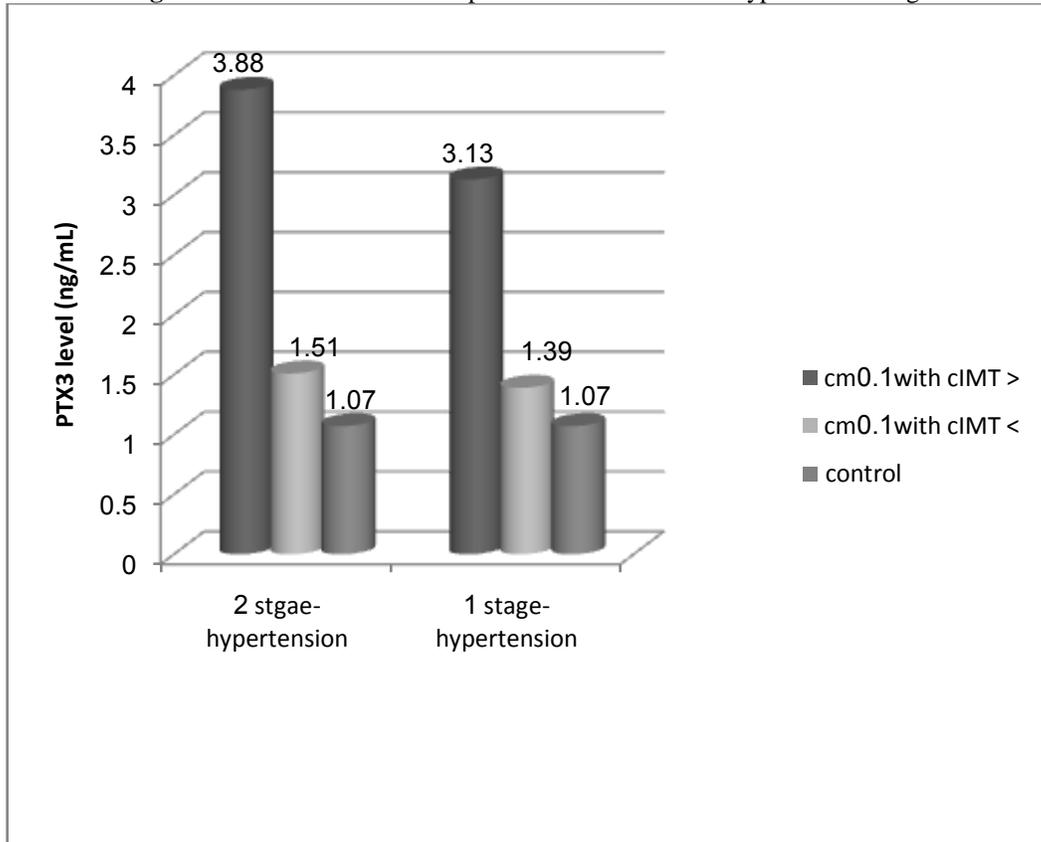
**Table 4:-** Correlation of plasma PTX3 levels with cIMT and hypertension stages.

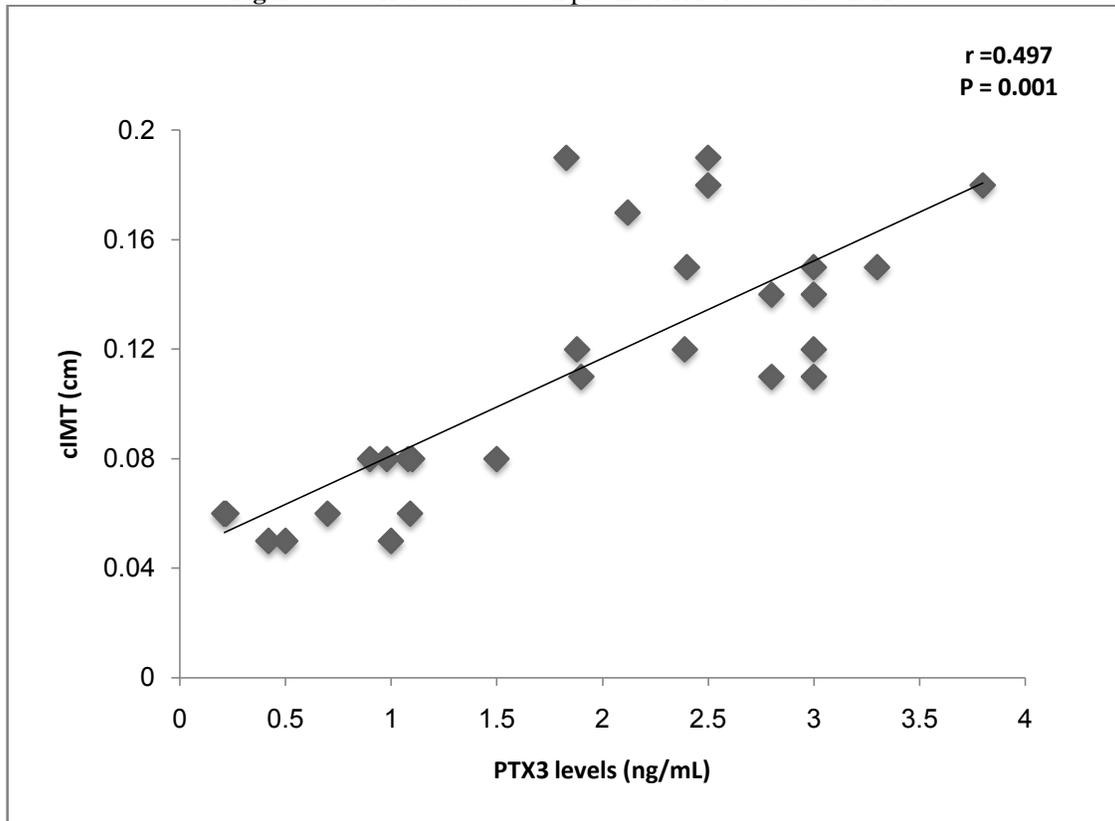
Item		PTX3 (ng/mL)	Statistical test	P value
cIMT	cIMT < 0.1cm	1.43± 0.37	T = 11.03	P < 0.001
	cIMT > 0.1cm	3.27 ± 0.61		
Hypertension	Stage-1 with cIMT < 0.1cm	1.39 ± 0.47	F = 50.37	P = 0.001
	Stage-1 with cIMT > 0.1cm	3.13 ± 0.82		
	Stage-2 with cIMT < 0.1cm	1.51 ± 0.59		
	Stage-2 with cIMT > 0.1cm	3.88 ± 1.01		
	Controls	1.07 ± 0.57		

**Figure 1:-** Correlation between plasma PTX3 levels and UACR (albuminuria).



**Figure 2:-** Correlation between plasma PTX3 levels and hypertension stages.



**Figure 3:-** Correlation between plasma PTX3 levels and cIMT.**Discussion:-**

PTX3, a long pentraxin, differs from classical short pentraxin in gene organization, cellular source, and ligands recognized. It is synthesized locally at the inflammatory sites by endothelial and smooth muscle cells or by monocytes/macrophages upon exposure to primary inflammatory signals such as IL-1, TNF- $\alpha$ , oxidized LDL, and bacterial products<sup>[5]</sup>. Association of biomarkers of systemic inflammation with atherosclerosis progression in carotid artery is not well established<sup>[18]</sup>. The current study aimed to assess plasma PTX3 levels in Egyptian patients with primary hypertension and to evaluate the value of plasma PTX3 level as a predictor for asymptomatic preclinical hypertensive-related atherosclerotic vascular disease. This study included 75 hypertensive patients, with a mean age of 54.7 $\pm$ 9.3 years (range, 38-73 years), in addition to 15 age- and sex-matched healthy subjects as a control group. In present study, mean plasma PTX3 level in control subjects was 1.07 $\pm$ 0.57ng/mL. Comparable results of PTX3 levels in controls were reported by other studies<sup>[5]</sup>.

Plasma PTX3 levels in current study correlated directly with the age of hypertensive patients, where older hypertensive patients had significantly higher plasma PTX3 levels. Similarly, plasma PTX3 levels correlated directly with patients' age<sup>[8]</sup>. Elevated plasma PTX3 levels could be a significant predictor of cardiovascular disease (CVD) in elderly hypertensive patients<sup>[12]</sup>. No significant differences were reported regarding plasma PTX3 levels between hypertensive males and females in our study. Contradictory, Nabrdalik et al,<sup>[18]</sup> reported higher plasma PTX3 levels in males compared to females and, in contrast, Abu Seman et al,<sup>[23]</sup> found lower plasma PTX3 concentration in males compared to females. This contradiction could be related to the studied patients, where our study included hypertensive patients while the study of Nabrdalik et al,<sup>[18]</sup> included type-2 diabetic patients and that of Abu Seman et al,<sup>[23]</sup> included type-2 diabetic patients with diabetic nephropathy.

In present study, significantly higher plasma PTX3 levels were reported among hypertensive patients with elevated LDL cholesterol ( $\geq$  100mg/dL) as well as among hypertensive patients with low HDL cholesterol (< 50mg/dL in males; < 40mg/dL in females). Jylhava et al<sup>[8]</sup> demonstrated that plasma PTX3 concentration correlated with several cardiovascular risk factors in individuals at higher risk of developing cardiovascular disease. Plasma PTX3 levels correlated directly with triglyceride levels and inversely with HDL cholesterol levels, suggesting an association

between PTX3 and atherogenic lipid<sup>[24]</sup>. It is postulated that lower PTX3 levels may be associated with the HDL cholesterol-induced PTX3 inhibition<sup>[15]</sup>. In hypertensive patients, higher degrees of UACR and albuminuria in our study were associated with significantly elevated plasma PTX3 levels, where hypertensive patients with macroalbuminuria had significantly higher plasma PTX3 levels compared to patients with microalbuminuria as well as to patients with normal urinary albumin levels, and hypertensive patients with microalbuminuria had significantly higher plasma PTX3 levels compared to patients with normal urinary albumin levels. In agreement, plasma PTX3 concentration was associated with elevated UACR<sup>[18]</sup>, and plasma PTX3 concentration was independently associated with proteinuria<sup>[25]</sup>.

In this study, plasma PTX3 levels showed no significant differences with stages of hypertension, where patients with stage-1 hypertension had no significant differences in plasma PTX3 levels compared to those with stage-2 hypertension; of note, patients with hypertensive crisis were not included in current study. In contrast, in hypertensive subjects, PTX3 correlated directly with diastolic blood pressure and pulse pressure<sup>[8]</sup>. Hypertensive patients had higher plasma PTX3 levels compared to normotensive subjects, and PTX3 could be considered as a novel biomarker for hypertension<sup>[26]</sup>. This contrast could be explained by the exclusion criteria of selected patients, where our study excluded patients with risk factors for atherosclerosis other than hypertension and dyslipidemia whereas other studies did not exclude patients with such risk factors.

In current study, presence of preclinical atherosclerotic vascular disease, as reflected by cIMT > 0.1cm, was associated with higher PTX3 levels, where hypertensive patients, either stage-1 or stage-2 hypertension, with preclinical atherosclerotic vascular disease had significantly higher PTX3 levels compared to hypertensive patients without preclinical atherosclerotic vascular disease as well as to controls. Concurrently, plasma PTX3 levels were significantly higher in patients with cardiovascular disease (CVD) compared to those without<sup>[5]</sup>. Also, PTX3, a novel marker of vascular disease, is higher in patients with subclinical atherosclerosis<sup>[24]</sup>. PTX3 has the potential to be a valuable tool not only in predicting severity of coronary artery disease but also in the prognosis of patients with coronary artery disease<sup>[20]</sup>.

In present study, plasma PTX3 levels had significantly positive-correlation with the cIMT, where higher plasma PTX3 levels were reported among patients with increased cIMT (> 0.1cm) and lower plasma PTX3 levels were reported among patients with normal cIMT (< 0.1cm). Consistently, positive association between cIMT and PTX3 levels were reported<sup>[5]</sup>. Significantly higher mean PTX3 levels were reported among patients with carotid atherosclerosis compared to those without<sup>[18]</sup>. Suliman et al,<sup>[25]</sup> found a correlation of plasma PTX3 concentration and albuminuria to be independently associated with carotid intima thickness. In contrast, plasma PTX3 concentration was not an independent predictor of progression of subclinical atherosclerosis in carotid and femoral arteries<sup>[18]</sup>. Moreover, the associations between plasma PTX3 and cIMT were not confirmed by other analyses<sup>[24]</sup>. This contradiction could be explained by that in addition to PTX3, other factors play an important role in promoting carotid artery intima-media thickness. Also, plasma PTX3 levels in Baragetti et al,<sup>[21]</sup> study were not associated with cIMT progression and plasma PTX3 level is neither an independent predictor of progression of subclinical atherosclerosis nor of incident cardiovascular events. The contrast between our results and that of Baragetti et al,<sup>[21]</sup> could be explained by the selected patients where our study included hypertensive patients whereas Baragetti et al,<sup>[21]</sup> study included general population.

### **Conclusion and Recommendation:-**

Elevated plasma PTX3 levels were reported in Egyptian hypertensive patients with asymptomatic preclinical atherosclerotic vascular disease, regardless of hypertension stages, and elevated plasma PTX3 levels were correlated with cIMT, an established marker of subclinical atherosclerosis. Further studies are recommended to put a cut-off point for plasma PTX3 level at which the presence of preclinical atherosclerotic vascular disease in hypertensive patients could be suggested.

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