

Original Research Article**Association of TNF-alpha with Blood Pressure levels in Prehypertensive Adults in Makurdi, Nigeria.****ABSTRACT**

Background: Previous studies have reported vascular inflammation in hypertension, which mediates its complications. However, studies' regarding vascular inflammation in prehypertensives has been neglected. **Aim:** Our study aims at investigating vascular inflammation using tumor necrosis factor-alpha (TNF- α) in prehypertensive individuals. **Materials and Methods:** This case-control study comprised of 70 randomly selected male and female patients aged 18-55 years, who were attending general health check up at the tertiary hospital. The biochemical and anthropometric parameters of 35 prehypertensives were compared with 35 normotensive control group. **Results:** Prehypertensives (systolic blood pressure [SBP] 130.91 ± 8.70 ; diastolic BP 83.17 ± 5.86) had significant ($P < .002$), elevated TNF- α level compared to anthropometrically matched normotensives (SBP 111.03 ± 6.89 ; DBP 70.40 ± 3.87). No significant difference ($P > 0.05$) was observed in the levels of lipids between the two study groups. A significant positive correlation was observed between; TNF- α and SBP ($r = .387$, $P = .022$), TNF- α and DBP ($r = .381$, $P = .024$) in prehypertensive group. There was no significant correlation ($P > 0.05$) between; TNF- α and plasma lipids, TNF- α and body mass index, TNF- α and waist circumference in prehypertensive group. **Conclusion:** Our study reveals a positive association of TNF- α with prehypertensive status. It is indicative that prehypertension predisposes individuals to mild chronic inflammation.

Keywords: Prehypertension, tumor necrosis factor-alpha, inflammation, blood pressure

1. INTRODUCTION

High blood pressure is among the risk factors of cardiovascular and renal diseases. A limited knowledge of high blood pressure (HBP) risk factors has exposed individuals to HBP [1]. The concept prehypertension (PreHTN) was introduced as a guideline for the management of blood pressure by the seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment (JNC 7) of HBP [2]. Prehypertension was defined as a systolic blood pressure of 120–139 mmHg and/or a diastolic blood pressure of 80–89 mmHg. Patients with PreHTN (120–139/80–89 mmHg) have an increased risk of cardiovascular morbidity and mortality compared with normotensive ($< 120/80$ mmHg) individuals [3]. The National Health and Nutrition Examination Survey (NHANES) 1999-2000 reported that the overall prevalence of preHTN was 31% all over the world. A statistical analysis of disease-free (apparently healthy) adult NHANES participants conducted from 1999 to 2006 found the overall prevalence of PreHTN in apparently healthy adults to be 36.3% [4].

Prehypertension is a precursor of clinical hypertension [5-7] and correlated with obesity, diabetes mellitus, dyslipidemia, which are recognized traditional cardiovascular risk factors [8-11]. Prehypertensive individuals are not usually placed on antihypertensive drug therapy, but rather advised to modify their lifestyle to lower their blood pressure to normal values targeted at reducing the risk of developing hypertension [12]. Patients with elevated arterial pressures and no definable cause are said to have Primary, Essential or idiopathic hypertension [13].

Vascular inflammation has been reported to play key roles in the pathogenesis and progression of hypertension [14]. Published data suggest that chronic inflammation could be an independent risk factor of high blood pressure [15]. Tumor necrosis factor-alpha (TNF- α) shown by several studies as a biomarker of mild chronic inflammation, is a proinflammatory cytokine synthesized by various cell types under the stimulatory action of cell stressors [16]. The primary inflammatory functions of TNF- α include; enhancement of cell differentiation, proliferation and apoptosis [17]. Most cardiovascular risk factors have been recognized to promote a pro-inflammatory state [18-20]. Arterial hypertension has been shown by previous studies to be independently related with circulating inflammatory markers [21-23].

Over the years researchers have paid more attention on the existence of generalized endothelial dysfunction in essential hypertension than preHTN. However, the presence of any relationship between preHTN status and inflammation has not been thoroughly investigated. Most previous studies regarding inflammation were conducted in hypertensives, which failed to control for other risk factors of high blood pressure. The purpose of this study was to investigate the relationship between preHTN status and inflammation, controlling for other risk factors of high blood pressure.

2. MATERIALS AND METHODS

2.1 Study Design

The case-control study compared the anthropometric and biochemical parameters of prehypertensives with normotensive controls.

2.2 Study Area and Population

A sample of male and female participants aged 18-55 years, was randomly drawn from patients attending general health checkup at the Federal Medical Centre Makurdi, from December 2015 to February 2016.

2.3 Sample size

The sample size was determined using the formula for case-control studies that compares two group means [14]; $n=1+2C(s/d)^2$, where s , the standard deviation (an estimate of the population standard deviation of blood pressure), d the effect size (the estimated mean difference of the blood pressure of prehypertensives and normotensives) and C a constant dependent on the level of significance and statistical power. At a significance level of 5%, and statistical power of 90%, C is 10.51. The calculated sample size was 32.4, which was approximated to 35 for each group.

2.4 Selection Criteria

Individuals were eligible to participate in the study if they: (a) were 18 to 55 years of age; (b) had no history of hypertension and were not using antihypertensive medications; (c) had no history of cardiovascular disease (coronary disease, stroke, peripheral vascular disease) and were not using lipid lowering drugs; (d) were free of any other major systemic illnesses (e.g. a form of cancer); (e) were nonsmokers; (f) were not pregnant. All subjects provided written informed consent, and the study was approved by the institutional ethical committee.

2.5 Data Collection

Participants on a 12 hour overnight fast, arrived during morning hours in a fasting state. Self-reported information on demographic characteristics (age, sex, financial status, and education), detailed medical history, dietary and lifestyle habits, such as smoking status, and physical activity were obtained.

2.5.1 Blood Pressure

All participants rested at least 30 min. Blood pressure measurements were taken using an aneroid manometric sphygmometer, three times with the right arm relaxed and well supported by a table, at an angle of 45-degree from the trunk. The systolic BP level was determined by the first perception of sound. The diastolic BP level was determined by phase V when the repetitive sounds become fully disappeared. Patients whose average BP levels were greater or equal to 140/90 mmHg or were taking antihypertensive medication or had a physician tell them that they have hypertension but were untreated were classified as hypertensives. Participants who had mean systolic/diastolic BPs within the range of 120 to 139/80 to 89 mmHg and never told that they had high BP levels were defined as prehypertensives, as it has been suggested by the JNC 7.^[2] The remainder of the participants were defined as normotensives.

2.5.2 Body mass index and waist circumference

Body weight to the nearest 0.1 kg and height to the nearest centimeter were measured with the subjects barefoot and in light clothing and BMI was calculated as weight (kilograms)/height (meters squared). Waist circumference (WC) was measured horizontally at the level of the natural waist, which was identified as the level at the hollow molding of the trunk when the trunk was laterally concave.

2.5.3 Blood sample collection

Blood samples were collected between 8 and 10 AM from fasting participants. Subjects were supine for 10 min before blood collection. Blood was collected into a plain glass vacutainer tube for serum lipids and TNF- α determination. All samples were collected without occlusion. The tubes were kept on ice until centrifuged at 3000 rpm for 10 min within 2 h of blood collection. The serum was extracted aseptically and stored at -70 °C for estimation of TNF- α . The biochemical evaluation using fresh serum was carried out in the laboratory of the institution following the criteria of the World Health Organization reference laboratories.

2.6 Laboratory Methods

The E-max microplate reader manufactured by Molecular Devices, Sunnyvale, USA, was used for immunoassay of TNF- α , whereas a spectrophotometer OPTIMA SP 300 manufactured by OPTIMA Inc., Tokyo, Japan, was used for analysis of fasting plasma glucose, total cholesterol, high density lipoprotein cholesterol (HDL-C), and triglycerides (TG).

2.6.1 Determination of serum tumor necrosis factor-alpha

The TNF- α immunoassay kit, obtained from DRG international California, USA, was used for the assay of TNF- α . The determination of TNF- α in serum was based on the enzyme linked immunosorbent assay sandwich principle.

2.6.2 Determination of serum lipids

The reagent kit for the determination of total cholesterol, HDL-C, and TG was obtained from Randox laboratories Limited, United Kingdom. Total cholesterol was determined by the cholesterol esterase method. The HDL-C was determined by the cholesterol esterase method after fractional separation from other lipids. Triglycerides were determined using the lipase method. LDL-C and VLDL-C were estimated using the Friedewald equation [24].

2.7 Statistical analysis

Continuous variables were presented as mean values $\pm$ standard deviation. Comparisons between continuous variables of prehypertensive and normotensive groups were performed by the student's t-test. Pearson's correlation coefficient was used in testing relationships between TNF- α and systolic BP, diastolic BP, total cholesterol, HDL-C, LDL-C, VLDL-C and TGs. All reported *P* values were based on two-sided tests and compared to a significance level of 5% (0.05). SPSS version 21 (Statistical Package for Social Sciences; IBM Armonk, New York, United States) software was used for all the statistical calculations.

3. RESULTS

The comparison of serum TNF- α level, total cholesterol, HDL-C, LDL-C, VLDL-C, TGs between prehypertensives and anthropometrically matched normotensives is presented in Table 1. Prehypertensives had significant ($P<0.002$), elevated TNF- α level compared to normotensives, with no significant difference ($P>0.05$) observed in the levels of lipids between the two study groups.

Figure 1 and 2 respectively presents the relationships between TNF- α and systolic BP, TNF- α and diastolic BP in prehypertensive subjects. A significant positive correlation was observed between; TNF- α and systolic BP ($r=0.387$, $P=0.022$), TNF- α and diastolic BP ($r=0.381$, $P=0.024$) in prehypertensive subjects.

Figure 3 and 4 respectively presents the relationships between TNF- α and systolic BP, diastolic BP in all the apparently healthy subjects studied. In the overall subjects studied, TNF- α level was shown to significantly correlate positively with both systolic BP ($r=0.473$, $P=0.000$), diastolic BP ($r=0.467$, $P=0.000$).

Table 2 presents the relationships of TNF- α , blood pressure with lipids, age, body mass index, waist circumference in prehypertensive subjects. There was a significant ($r=0.414$, $P=0.013$) relationship between TNF- α and age, where as no significant correlation ($P>0.05$) was observed between; TNF- α and lipids (total cholesterol, HDL-C, LDL-C, VLDL-C, TGs), TNF- α and BMI, TNF- α and waist circumference in prehypertensive subjects. There was no significant correlation ($P>0.05$) between; blood pressure (systolic & diastolic) and lipids (total cholesterol, LDL-C, VLDL-C, TGs), blood pressure and age, blood pressure and BMI, blood pressure and waist circumference in prehypertensive subjects.

The relationships of TNF- α , blood pressure and lipids, age, body mass index, waist circumference in the overall subjects studied is presented in Table 3. Where as a significant ($r=0.326$, $P=0.006$) correlation existed between TNF- α and age, a non significant correlation ($P>0.05$) was observed between; TNF- α and lipids (total cholesterol, HDL-C, LDL-C, VLDL-C, TGs), TNF- α and BMI, TNF- α and waist circumference) in all the apparently healthy subjects. No significant correlation ($P>0.05$) was observed between; blood pressure (systolic & diastolic) and lipids (total cholesterol, LDL-C, VLDL-C, TGs), blood pressure and age, blood pressure and BMI, blood pressure and waist circumference in all the apparently healthy subjects.

Table 1. Blood pressure, age, BMI, waist circumference, TNF- α , Lipids in prehypertensive and normotensive control subjects

Parameters	PreHTN (n=35)	Control (n=35)	Calc. t- value	P-value
SBP (mmHg)	130.91 $\pm$ 8.70	111.03 $\pm$ 6.89	10.60	0.000*
DBP (mmHg)	83.17 $\pm$ 5.86	70.40 $\pm$ 3.87	10.80	0.000*
Age (years)	35.57 $\pm$ 10.73	33.63 $\pm$ 9.10	0.82	0.417
BMI (Kg/m ²)	24.72 $\pm$ 3.39	24.40 $\pm$ 3.51	1.81	0.072
WC (cm)	69.17 $\pm$ 8.04	68.20 $\pm$ 5.96	0.40	0.694
TNF- α (pg/mL)	52.85 $\pm$ 90.69	4.59 $\pm$ 5.08	3.14	0.002*
T.Chol (mmol/L)	4.28 $\pm$ 0.63	4.18 $\pm$ 0.58	0.71	0.483
HDL-C (mmol/L)	1.61 $\pm$ 0.37	1.59 $\pm$ 0.37	0.19	0.848
LDL-C (mmol/L)	2.21 $\pm$ 0.70	2.14 $\pm$ 0.70	0.45	0.658
VLDL-C (mmol/L)	0 .49 $\pm$ 0.22	0.45 $\pm$ 0.13	1.10	0.274
TG (mmol/l)	1.00 $\pm$ 0.38	0.94 $\pm$ 0.30	0.77	0.445

mean $\pm$ standard deviation, critical $t = 1.99$, *significant, SBP- systolic blood pressure, DBP- diastolic blood pressure, WC- waist circumference, BMI- body mass index, T.Chol.-total cholesterol, HDL-C- high density lipoprotein cholesterol, LDL-C- low density lipoprotein cholesterol, VLDL-C- very low density lipoprotein cholesterol, TG- triglyceride

Table 2. Pearson's correlation coefficients of blood pressure and TNF-alpha with other variables in prehypertensive subjects.

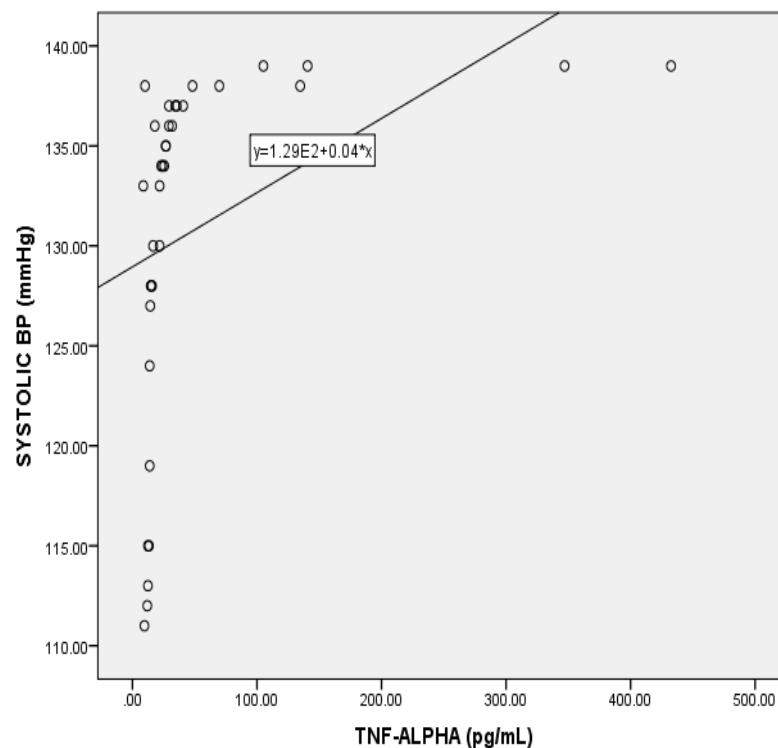
Parameters	Age	BMI	WC	TC	HDL	LDL	VLDL	TG
SBP	0.224	-0.033	-0.065	-0.077	0.441*	-0.261	0.038	-0.145
DBP	0.128	0.162	-0.027	0.142	0.412*	-0.045	-0.055	-0.144
TNF-α	0.414*	0.115	-0.167	0.297	0.260	0.065	0.170	0.221

* Correlation is significant at the 0.05 level (2-tailed). SBP- systolic blood pressure, DBP- diastolic blood pressure, WC- waist circumference, BMI- body mass index, TC- total cholesterol, HDL- high density lipoprotein cholesterol, LDL- low density lipoprotein cholesterol, VLDL- very low density lipoprotein cholesterol, TG- triglyceride

Table 3. Pearson's correlation coefficients of blood pressure and TNF-alpha with other variables in overall apparently healthy subjects.

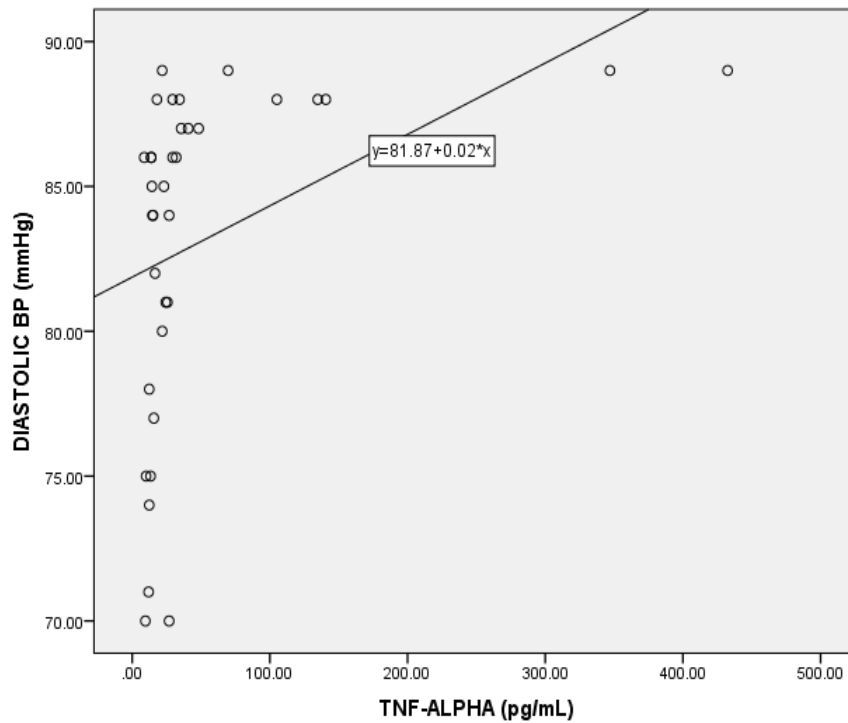
Parameters	Age	BMI	WC	TC	HDL	LDL	VLDL	TG
SBP	0.146	0.023	-0.001	-0.009	0.157	-0.088	0.109	0.030
DBP	0.208	0.221	0.111	0.133	0.134	0.039	0.143	0.093
TNF-α	0.326**	0.086	-0.107	0.229	0.182	0.056	0.185	0.195

**Correlation is significant at the 0.01 level (2-tailed), SBP- systolic blood pressure, DBP- diastolic blood pressure, WC- waist circumference, BMI- body mass index, T.Chol- total cholesterol, HDL-C- high density lipoprotein cholesterol, LDL-C- low density lipoprotein cholesterol, VLDL-C- very low density lipoprotein cholesterol, TG- triglyceride



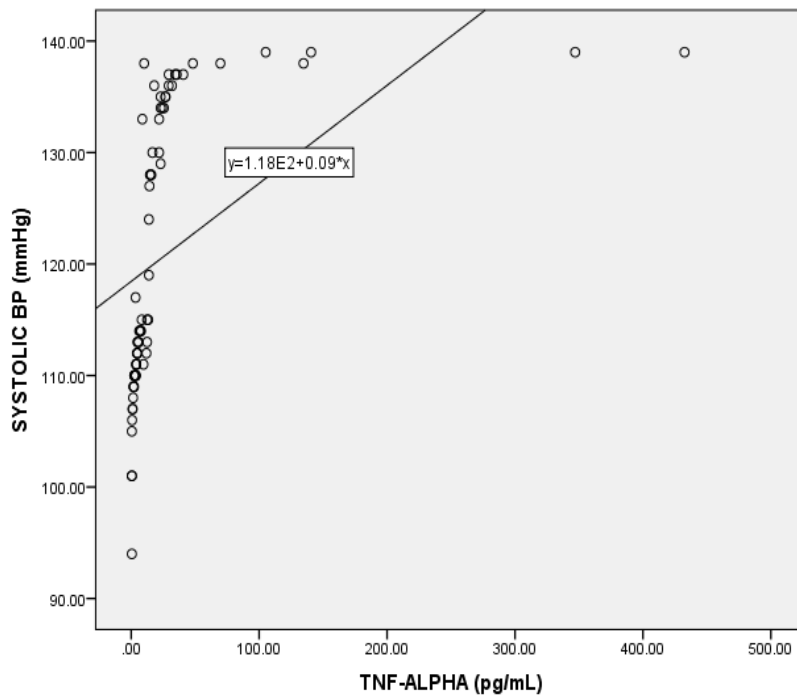
$r=0.387$, $n=35$, $p=0.022$, BP- blood pressure, TNF-tumor necrosis factor

Fig. 1. Correlation of TNF-alpha with systolic blood pressure in prehypertensive subjects



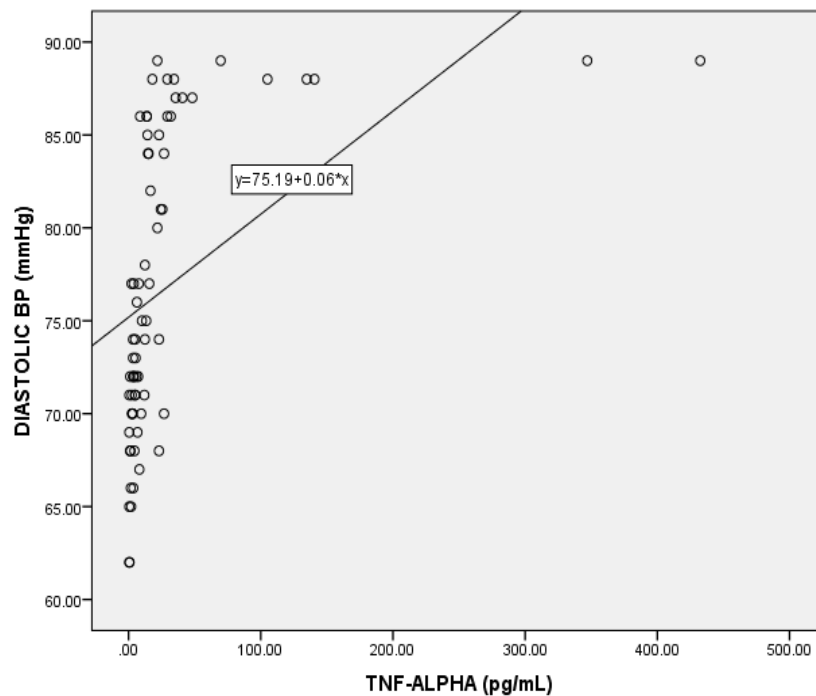
$r=0.381$, $n=35$, $p=0.024$, BP- blood pressure, TNF-tumor necrosis factor

Fig. 2. Correlation of TNF-alpha with diastolic blood pressure in prehypertensive subjects



$r=0.473$, $n=70$, $p=0.000$, BP- blood pressure, TNF-tumor necrosis factor

Fig. 3. Correlation of TNF-alpha with systolic blood pressure in all apparently healthy subjects



r=0.467, n=70, p=0.000, BP- blood pressure, TNF-tumor necrosis factor

Fig. 4. Correlation of TNF-alpha with diastolic blood pressure in all apparently healthy subjects

4. DISCUSSION

Prehypertension is a precursor of clinical hypertension. Hypertension is a well known major injurious risk factor that promotes cardiovascular diseases like atherosclerosis. Vascular inflammation is the hallmark in the pathogenesis and progression of hypertension and atherosclerosis. Our study determined circulating TNF- α as a biomarker of mild chronic inflammation in prehypertensives. Previous studies on inflammation have laid much emphasis on hypertensive patients neglecting prehypertensives. It is in this light that our study sought to investigate the relationship between mild chronic inflammation and preHTN independent of confounding factors of high blood pressure.

Our study compared TNF- α level, lipids in anthropometrically matched prehypertensive and normotensive apparently healthy subjects. In this study, while lipid levels remained the same, TNF- α level was observed to be higher in the prehypertensive than normotensive individuals. This study, found a positive correlation of TNF- α with blood pressure (systolic and diastolic) separately in prehypertensives and overall apparently healthy participants. Blood pressure (systolic and diastolic) did correlate with neither lipids nor physical variables in prehypertensives and the overall participants

of our study. Exception of age, TNF- α levels neither correlated with lipids nor physical variables in prehypertensives and the overall participants of our study.

This study is consistent with some studies on inflammation, conducted in hypertensive subjects. Increased plasma concentration of proinflammatory cytokines, in hypertensive individuals have been confirmed in such studies [25-28]. A previous study conducted in different grades of blood pressure in essential hypertensive patients, show that TNF- α level were consistently found to be elevated in both stage 1 and 2 of hypertension [13]. Daytime diastolic blood pressure has been shown to be independently associated with TNF- α in hypertensive patients [29,1]. In apparently healthy Japanese women, Ito et al. found an association of serum TNF- α with serum low-density lipoprotein-cholesterol and blood pressure [30]. Chae et al. reported a significant association between inflammatory markers and elevated BP in apparently healthy patients [31]. Abramson et al. demonstrated a positive association between TNF- α and daytime diastolic BP in healthy, normotensive adults [32]. Chrysohoou et al. studied the association between prehypertension status and inflammatory markers related to atherosclerotic disease and observed a higher increase in TNF- α level, in prehypertensive subjects compared to normotensive subjects [33].

The studies of Sheu et al. and Mendall et al. in human subjects did not agree with the positive association between TNF- α level and blood pressure found in our prehypertensive study and hypertensive studies of others [34,35].

Essential hypertension is characterized by increased peripheral vascular resistance to blood flow that induces stress on the vascular endothelium contributing to complications of hypertension [36,37]. In hypertension, resistance arteries undergo a process of inflammatory remodeling involving extracellular matrix deposition and chronic vasoconstriction [37,38]. Experimental data suggest that elevated blood pressure may stimulate a pro-inflammatory response resulting to endothelial inflammation in the arterial walls that characterize the hypertensive state [39,40]. There is some experimental evidence suggesting that elevated BP may specifically promote endothelial expression of cytokines that stimulate inflammation associated with hypertension [31,41]. An experimental study conducted by Fernandez-Real et al. showed that the degree of activation of the TNF- α system was positively and significantly associated with systolic and diastolic BP in type 2 diabetics [42]. TNF- α has been shown to decrease endothelial nitric oxide synthase mRNA level by shortening its half-life [43]. This could decrease bioavailable nitric oxide leading to endothelial dysfunction, followed by

chronic vasoconstriction, and elevated blood pressure. Also, a polymorphism in the promoter region of the TNF- α gene has been associated to increased TNF- α and systolic BP [44]. There is a possibility that elevated TNF- α , a marker of mild chronic inflammation could initiate the development of high blood pressure, or enhance the progression of hypertension through stages to complications.

5. CONCLUSION

Our study reveals an association of prehypertensive status with TNF- α . It is indicative; prehypertension predisposes individuals to mild chronic inflammation that mediate progression to hypertension and its complications. This implies that moderate rise in blood pressure could also serve as a stress inducer of TNF- α production in initiating vascular inflammation. We recommend the adoption of healthy lifestyles geared towards maintenance of normal blood pressure levels.

CONSENT

The patient's written consent has been collected and preserved by the authors.

ETHICAL APPROVAL

Ethical approval for this study was obtained from the ethics committee of Federal Medical Centre, Makurdi, Nigeria

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