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Role of TNF-α in hypertension and its modulation by antihypertensive agents

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Abstract

Background: Hypertension is the leading cause of cardiovascular disease and contributes to10 million deaths per year. It exerts an enormous public health burden on cardiovascular health status and healthcare systems in India. There is up growing evidence that inflammation and abnormal immune responses are involved in the etio-pathogenesis of hypertension but role of any specific pro-inflammatory cytokine is controversial.
Objectives: 1) To compare the levels of TNF-α in treatment naïve hypertensive patients with those on regular anti-hypertensive medications for at least three months. 2) To assess whether change in TNF-α levels corresponds to change in BP
Methods: The study included 3 groups of 30 subjects each (Group 1- Known cases of essential hypertension on regular anti-hypertensive medications for at least 3 months, Group 2-treatment naïve hypertensive patients, Group 3 – normotensive controls) of either sex in the age group 30-70 years who attended the OPD, Department of Medicine at a tertiary care hospital of Northern India. Written informed consent was taken from all the study participants. Blood samples were collected from every patient and TNF-α levels were determined using Human TNF-α ELISA kit.
Results: TNF- α levels were statistically significant between the 3 groups ($p < 0.001$).TNF-α value in the Group 1 had a mean of 18.94 ± 7.01 pg/mL, Group 2 had a mean of 29.83 ± 10.03 pg/mL and Group 3 had a mean of 9.29 ± 6.44 pg/mL.
Conclusion: 1) TNF- α was significantly higher in patients with hypertension as compared to normotensive subjects. 2) TNF-α in patient on antihypertensive medication was significantly less than patients who were treatment naïve.
Keywords: Hypertension, pro-inflammatory cytokines, TNF- α, Antihypertensive medication.

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1. Introduction

Hypertension is the leading cause of cardiovascular diseaseand is a public health concern. It contributes to10 million deaths per year[1]. 1.13 billion people around the world have reported with hypertension and the numbers are expected to increase to 1.5 billion by 2025[2].

Over the last 25 years there is an increasing prevalence of hypertension among Indian urban population[3]. Recent epidemiological studies have reported that hypertension is present in 25–30% urban and 10–20% rural subjects in India[4,5]. It is estimated that the burden of hypertension in India is expected to almost double from 118million in 2000 to 213.5million by 2025[5].

Treatment of hypertension responds to once a day, cost-effective anti-hypertensive medications which are both safe and efficacious. Nevertheless, only less than 15% of adults with hypertension worldwide have their blood pressure controlled to 140/90 or lower [6].

Various mechanisms are implicated in the etio-pathogenesis of hypertension. There is increasing evidence that inflammation and abnormal immune responses are involved in the pathogenesis of hypertension[7]. Hypertension is considered to be a low-grade inflammatory disorder characterized by the presence of various pro-inflammatory cytokines. Tumor necrosis factor-α (TNF-α) is an important constituent of the pro-inflammatory cytokines. Elevated angiotensin II (ANG II) and other factors such as oxidative stress conditions promote TNF-α formation. Recent evidence indicates that TNF-α also exerts a direct renal action by regulating hemodynamic and excretory function in the kidney. The cytokine triggers a strong natriuretic response and plays a significant role in the regulation of the intra-renal renin-angiotensin system[8].

Experimental evidence suggests Renin Angiotensin System (RAS) to play a crucial role in the pathogenesis of hypertension and hypertension related end-organ damage, but the role of any specific inflammatory cytokine is still controversial[9].

The scan of literature reveals few studiesregarding the role of anti-hypertensives on TNF levels in Indian population. Hence, the current study was undertaken to evaluate the modulation of TNF -α levels by anti-hypertensive medications in patients with hypertension and to compare the levels of TNF-α in treatment naive hypertensive patients with those on anti-hypertensive medications for at least three months and to evaluate whether any change in TNF-α levels correspond to change in BP.The results of the study can supplement the earlier work and shed more light on the subject.

2. Materials and Methods

A cross-sectional observational study was conducted in Post Graduate Department of Pharmacology in collaboration with the Department of Internal Medicine at a Tertiary Care Teaching Hospital at Greater Noida, Uttar Pradesh.The study protocol was approved by the Institutional Ethics Committee.Written informed consent was obtained from all the study subjects after explaining them the nature and purpose of study. All principles of Bioethics were followed.

A total of121patients attending the Medicine OPD were enrolled for the study. Out of these90 patients fulfilled the eligibility criteria and were subsequently included in the study.

The study population consisted of three different groups. The subjects were patients attending the Medicine OPD of Sharda Hospital, Greater Noida (U.P.) on regular follow-up or presenting with uncontrolled/unsatisfactorily controlled blood pressure. Group 1 included patients with essential hypertension on regular anti-hypertensive medication for a minimum of 3 months, Group 2 included treatment naïve patients with hypertension and Group 3 included normotensive healthy controls. Participants of either sex, age ≥ 30 to ≤ 70 years were included in the study.

Treatment naïve patients were defined as newly diagnosed patients of essential hypertension or patients not on anti-hypertensive medication for atleast last 3 months.

The exclusion criteria included, patients with secondary causes of hypertension, smokers,patients with history of autoimmune or inflammatory disorders like rheumatoid arthritis, inflammatory Bowel Disease, Psoriasis etc., patients with history of renal diseases, patients on anti-inflammatory medicationsthat could affect plasma levels of inflammatory markers in the last 2 weeks, history of any febrile illness or major trauma during the last 15 days before enrollment, pregnant or lactating women.

Clinical evaluation with complete medical history, general physical examination and systemic examination was done.

Anthropometric measurements were carried out and the mean of the two measurements was used during the analysis.BP was measured in the seated position, in the right arm, after a 5-min rest period, following the recommendations of the American Heart Association[10]. Hypertension was defined as per the JNC-8 criteria.

Blood samples were collected from each patient and serum was extracted from the samples by centrifugation at 1000g for 20 minutes after which it was stored in aliquot at -70°C for later use. The samples were tested for TNF-α levels by using Diaclone Human TNF-α ELISA test kits. The colour change in the vials indicated the TNF-α levels which was measured spectrophotometrically in a ELISA reader.

2.1 Statistical Analysis

The sample size was calculated with the following data: significance level of 0.05, SD of the dependent variable (TNF-α) 0.6, SD of the independent variable (HTN) = 0.06.Based on the statistical analysis the present study included 30 known cases of essential hypertension on regular anti-hypertensive medication for at least 3 months (Group 1), 30 treatment naïve patients with hypertension(Group 2)and 30 controls of either sex, age ≥ 30 to ≤ 70 years were included in the study who attended the Department of Medicine of SMSR Medical College and Sharda Hospital, Greater Noida, U.P. India.

All the data was collected, compiled and analyzed using Excel 2013. Data is presented as Mean ± SD. Pre and post treatment continuous variables were analyzed by ANOVA. A p-value of <0.05 was considered as statistically significant.

3. Observations and Results

A total of 121 patients were enrolled for the study. Out of these 90 patients fulfilled the eligibility criteria and were subsequently included in the study.

Table 1: Shows the baseline characteristics in the three groups

Parameter	Treated (Group 1)	Treatment naive (Group 2)	Control (Group 3)
Male : Female ratio	4:1	3.32:1	2:1
Age (years)	49.7 ± 12.7	50.3 ± 13.11	45.5 ± 8.80
Systolic BP (mmHg)	126.83 ± 13.45	151.66 ± 15.69	114 ± 7.14
Diastolic BP (mmHg)	81.66 ± 7.46	94.33 ± 6.79	76 ± 4.98
Height (cm)	162 ± 11.20	161.2 ± 13.72	163.3 ± 13.76
Weight (kg)	75.33 ± 12.23	74.8 ± 11.59	71.03 ± 11.76
BMI (kg/m^2)	28.7 ± 3.83	28.8 ± 4.08	26.6 ± 5.74

The subjects included in the study had a mean age of 48.5 ± 11.76 with a male: female ratio of 2.9:1.

The subjects in Group 1 had a mean systolic BP of 126.83 ± 13.45 mm of Hg, and the mean diastolic BP was 81.66 ± 7.46 mm of Hg. The TNF-α values in the drug treated group (Group 1) has a mean of 18.94 ± 7.01 pg/mL.

Similarly, the subjects in Group 2 had a mean systolic BP of 151.66 ± 15.69 mm of Hg, and the mean diastolic BP was 94.33 ± 6.79 mm of Hg. The TNF-α values in the treatment naïve patients (Group 2) has a mean of 29.83 ± 10.03pg/mL.

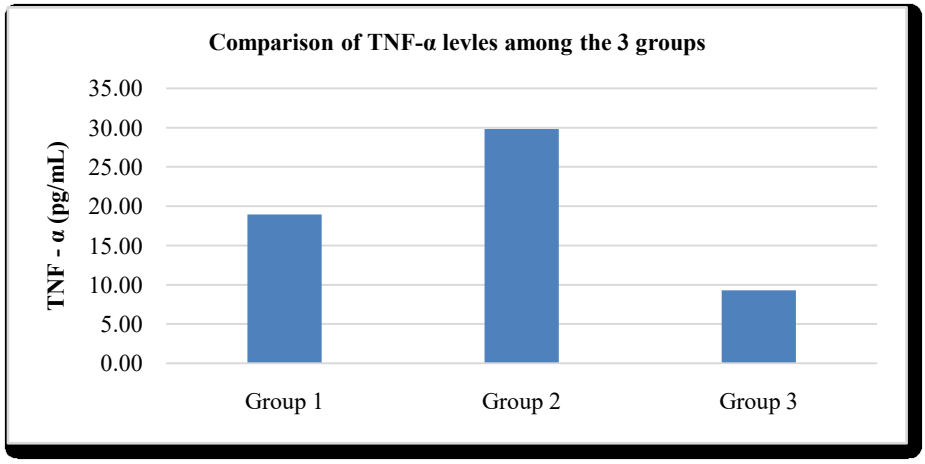
Controls in Group 3 had a mean systolic BP of 114 ± 7.14 mm of Hg, and the mean diastolic BP was76 ± 4.98 mm of Hg. The TNF-α values in the same(Group 3) has a mean of 9.29 ± 6.44pg/mL.

Table 2, Figure 1 shows the comparative values of TNF-α in the 3 groups

Table 2: Comparison of TNF-α between the 3 groups:

Group	TNF – α levels (Mean ± SD) in pg/mL	p-value
Group 1	18.94 ± 7.01	<0.0001
Group 2	29.83 ± 10.03	<0.0001
Group 3	9.29 ± 6.44	<0.0001

Figure 1 Comparison of TNF-α between the 3 groups:



4. Discussion

There is a rise in the estimates of global prevalence of hypertensionespecially in low- and middle- income countries [2,11]. It is one of the foremost causes of premature death worldwide[2].

In India overall prevalence for hypertension was reported to be 29.8%[4]. In an analysis of worldwide data for the global burden of hypertension, 20.6% of Indian men and 20.9% of Indian women were affected from hypertension in 2005[12]. The rates for hypertension in percentage are projected to go up to 22.9 and 23.6 for Indian men and women, respectively by 2025[12].

Hypertension is causative for 57% of all stroke deaths and 24% of all coronary heart disease deaths in India[13]. Pharmacotherapy with anti-hypertensive medications is the standard treatment. In spite of armamentarium of anti-hypertensive medications available in the market, it is reported only about 25.6% of treated patients had their BP under control[14].

Robust evidence suggests that low grade inflammation may have important implications in the development of hypertension[15]. However, because of the availability of limited scientific evidenceespecially in the Indian clinical setting, we sought to investigate the relationship between TNF-α and hypertension in a cross-sectional study at a tertiary care hospital of Northern India.

The present study was conducted with the aim of assessing the association between hypertension and TNF-α.

The rationale for assessing these parameters was to understand the relationship between hypertension and one of the pro-inflammatory mediators TNF-α.

The analysis of our study data showed male: female ratio of subjects was2.9:1. The total number of females was 23 and the total number of males was 67. The results are comparable to that observed in the earlier studies. A study conducted by Mills *et al* also reported similar results where age-standardized prevalence of hypertension was slightly higher in men than in women[11].

The hypertension commonly occurs in middle aged group (especially after 40 years of age). The mean age of patients in the present study was 48.5 ± 11.76 years, reflecting the standard age group of disease manifestation. This was comparable to the age of the patients in two India specific studies where it was reported to be 53.9 years and 54.2 years[13].

It was seen that average body mass index (B)MI was not in the normal range across Group 1 and Group 2 suggesting that most of our patients were overweight or obese. Overweight and obesity are well known independent risk factors for the development of hypertension and progression of the disease[4,16]. So, as expected, patients with hypertension were significantly older andhad a significantly higher BMI (Table 1).

The value of TNF-α in Group 1 was 18.94 ± 7.01 pg/mL which was significantly lower than Group 2 having TNF-α levels of 29.83 ± 10.06 ($p<0.0001$)indicating some kind of modulation of the serum TNF-α levels in the Group 1 by the regular intake of the anti-hypertensive medication. Group 3 including the normotensive healthy subjects had the lowest TNF-α levels of 9.29 ± 6.44 pg/mL.The results are consistent with the findings of Bautista *et al* who demonstrated that TNF-α levels are elevated in subjects with hypertension and decreased as a person reaches the normotensive goal. Other studies have also reported significant higher levels of TNF- α in hypertensivepatients than in normotensive individuals.The study shows a significant association between TNF-α levels and hypertension which is in accordance with the results of other studies[17,18].

Interestingly, the systolic and diastolic BP of the subjects in the three groups followed the same pattern as TNF-α levels. The highest levels were seen in the treatment naïve hypertensive subjects and lowest in the normotensive healthy controls. The results are in step with previous studies which have shown a positive association between TNF- α level and hypertension[19,20].

Several mechanisms have been postulated to elucidate the role of inflammatory cytokines in the development of hypertension. A key component could be the imbalance between endothelium-derived relaxing and contracting factors such as nitric oxide, endothelium- derived hyperpolarizing factor, and prostacyclin. Further, it has been postulated that TNF-α decreases endothelial nitric oxide synthase mRNA level by shortening its half-life[21].This could result in reduced bioavailability of nitric oxide and lead to endothelial dysfunction, chronic vasoconstriction and elevated BP. Finally, a common polymorphism in the promoter region of the TNF-α gene has been associated with increased TNF-α and systolic BP[22].Oxidative stress may also be involved, since reactive oxygen species can cause vasoconstriction both by decreasing nitric oxide and by increasing prostaglandins.

Another mechanism by which inflammation and cytokines may affect blood pressure is through increased arterial stiffness. Several observational studies have shown a relationship between the two, but still no conclusive data could be drawn and thus further studies are essential to understand this relationship[23].

The study subjects did not report any side effects other than what are expected from the anti-hypertensive drugs.

There are some limitations within the present study such as being cross sectional in nature. Since the study indicated that there is some modulation of the TNF-α levels by the anti-hypertensive medications, a more detailed analysis of the various anti-hypertensive medication classes could have thrown more insight as different drugs exert anti-inflammatory effects additionally to their antihypertensive properties, with improvement of cardiovascular outcome by reducing vascular inflammation and remodeling[15]. The number of patients was small; hence it might not be justified to extrapolate the results of the current study to the large population of patients with essential hypertension.Since blood samples for TNF- α levels were measured at the same point in time as BP, it is notpossible to know whether one precedes the other or vice versa.

5. Conclusion

The present work was undertaken to study the association of TNF-α with respect to hypertension. Hypertensive patients on anti-hypertensive medications and treatment-naïve patients were evaluated against normotensive controls and their TNF-α levels were measured.

TNF- α was significantly raised in patients with hypertension when compared to normotensive subjects. The levels were significantly less in patients on regular antihypertensive medicationsthan treatment naïve hypertensive patients indicating some kind of modulation of the serum TNF-α levels by the regular intake of the anti-hypertensive medications. Nevertheless, the temporal

relationship between serum TNF and hypertension should be further ascertained in prospective cohort studies.

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