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SELECTED MARKERS OF OXIDANT CHALLENGE IN ESSENTIAL HYPERTENSIVE SMOKERS

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ABSTRACT

Aim: The study was set to assess the relationship between antioxidant status, smoking and hypertension, the argument being whether there would be a significant difference in antioxidant status and perhaps, a significant difference in blood pressure.

Methods: A total of 105 samples were collected. 36 samples were collected from type I hypertensive smokers; 47 from hypertensive non smokers, the remaining 22 were collected from normal non smokers, who served as control. The blood pressure of each subject was measured. Determinations of SOD activity and TBARS content were carried out on each of the samples. Determination of systolic and diastolic blood pressure was also carried out using a digital sphygmomanometer. The results of all investigations were thereafter subjected to statistical analysis using SPSS 17, the student's t test being the tool of choice. Significance was tested at $P < 0.05$.

Results: The mean systolic and diastolic blood pressure of hypertensive smokers and non smoking hypertensives was found to be significantly increased. Similarly the mean systolic and diastolic blood pressure of hypertensive smokers was found to be significantly increased. SOD activity was significantly decreased while TBARS levels were significantly higher in both hypertensive groups. Lipid peroxidation was significantly higher while SOD activity was significantly lower in hypertensive smokers when compared with hypertensive non smokers

Conclusion: It appears that cigarette smoking as a social lifestyle depletes SOD levels but increases lipid peroxidation. It also seems to favour the progression of essential hypertension from mild to severity.

Keywords: Smoking, Hypertension, Reactive oxygen species, Antioxidant enzymes

INTRODUCTION

Hypertension (HTN) or high blood pressure (HBP) is a chronic medical condition in which the blood pressure in the arteries is elevated. It is classified as either primary (essential) or secondary. Majority of cases are termed primary or idiopathic hypertension, which refers to high BP for which no medical cause can be found (Carretero and Oparil, 2000). The remaining 5 to 10% of cases, called secondary HTN, are caused by other conditions that affect the kidneys, arteries, heart, or endocrine system (Beever et al., 2001). Persistent HTN is one of the risk factors

for strokes, heart attacks, heart failure, and arterial aneurysm, and is a leading cause of chronic kidney failure (Pierdomenico et al., 2009). Cigarette smoking on the other hand is a social habit that has been described as a lifestyle Factor that affects the health of humans (Alharbi, 2012). The habit of tobacco smoking starts during the period of adolescence or early adulthood as teenagers are attracted more by their peers than by the adults (Harris, 1998). There are numerous harmful substances found in tobacco and tobacco smoke (Proctor, 2012). The Cigarette smoke reaches quickly to heart, brain

and other parts of our body, and may cause effects in less than a second as it is inhaled directly into the alveoli and is diffused into the pulmonary vein (Alharbi, 2012). Reactive oxygen species (ROS) can be defined as reactive derivatives of O_2 metabolism. They exist in the environment and in all biological systems. In healthy conditions, ROS are produced in a controlled manner at low concentrations and function as signaling molecules (Touyz and Schiffrin, 2004). Important ROS detectable within the vasculature of human beings include the superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (OH^\cdot), and the reactive nitrogen species peroxynitrite ($ONOO^\cdot$), which have been regarded as a nasty, life-threatening, and destructive oxygen-derived toxicant (Virdiset al., 2012). In ideal conditions, ROS generation is tightly regulated by endogenous cellular antioxidants, which include superoxide dismutase (SOD), catalase, thioredoxin, glutathione, and antioxidant vitamins. In physiological conditions, the rate of ROS generation is counterbalanced by the rate of elimination. In contrast, under pathological conditions, ROS are produced in concentrations that cannot be matched by protective antioxidant mechanisms in the cells, when this happens, a state of oxidative stress ensues (Landmesser and Harrison, 2001). This research is therefore designed to assess the effect of smoking on markers of oxidant status and then, the clinical effect, if any, on existing hypertension.

MATERIALS AND METHODS

Inclusion and Exclusion Criteria: The criteria of selection of subjects (either smoking or non-smoking hypertensives) were that no one should have any clinical condition which could result in hypertension such as diabetes or any other disorder. Hence, all subjects included in the present study are type I hypertensives.

Blood samples: Fasting blood samples (10 ml) were collected from subjects and controls under aseptic precautions by Venepuncture. 5 ml of the blood samples were placed in heparinized tubes for SOD estimations, the remaining 3.0 ml was dispensed into a plain tube for serum Thiobarbituric acid reactive substances

(TBARS) determination. It was allowed to clot. Samples were centrifuged as soon as possible at 12000 rpm for 10 min at $4^\circ C$. Serum samples for TBARS were stored at $-70^\circ C$ until the time of analysis. After separating the plasma from the heparinized tubes, erythrocytes were washed three times in normal saline and were hemolysed by dilution in water and stored at $-20^\circ C$ until used for measurement of SOD and CAT activities

Determination of Biochemical Parameters

TBARS as a marker for lipid peroxidation and therefore oxidative stress, was determined using the thiobarbituric acid (TBA) method of Okhawa et al., (1979).

Erythrocyte SOD activity was assessed according to the method of Marklund and Marklund, (1979) which is based on the ability of SOD to inhibit auto-oxidation of pyrogallol. One unit of SOD being the activity of enzyme required to inhibit the auto-oxidation of pyrogallol by 50% in the assay mixture. The results are expressed in units/gHb.

Blood pressure was measured using a digital sphygmomanometer (Omros, Japan) according to manufacturer's guidelines.

Determination of blood pressure

The subjects were instructed to abstain from exercise, smoke or consumption of foods or drinks containing caffeine (such as tea or coffee) for at least 30 minutes before measurement. Blood pressure was done in duplicates at an interval of at least one minute between readings. The average value of the two readings was calculated. This value is taken to be the systolic and diastolic blood pressure. In case of the two readings on a subject differing by more than 5 mmHg, one additional reading was obtained before the average was taken.

Statistical analyses: Results were expressed as mean and standard deviation (SD). Statistical analysis was carried out using the SPSS program. All values are expressed as mean \pm SD and were found to be significant or otherwise at $P < 0.05$ (version 17.0 software, SPSS Inc. Chicago, Illinois, USA)

RESULTS

Table 1: Population, age, sex and values(mean±SD) of all estimated parameters

Parameters	Group I Hypertensive smokers	Group II Hypertensive Non smokers	Control Healthy non smokers
Population	36	47	22
Age(Years)	43.11 ± 6.71	47.24 ± 11.19	35.27 ± 8.39
Sex (f/m)	0/36	8/39	5/17
TBARS (nmol/L)	4.46 ± 1.13	3.62 ± 0.81	1.98 ± 0.47
SOD(U/gHb)	931.66 ± 188.12	1198.47 ± 125.19	1491.04 ± 164.83
Diastolic BP	97.72 ± 10.07	94.19 ± 13.61	77.32 ± 6.73
Systolic BP	149.81 ± 14.72	142.15 ± 11.34	116.29 ± 5.16

Table2: Hypertensive smokers versus control

Variables	Hypertensive Smokers	Control	Student's t (Critical t= 2.0032)	P value
TBARS(nmol/ml)	4.46 ± 1.13	1.98 ± 0.47	9.7642	.0000
SOD(U/gHb)	931.66 ±188.12	1491.04 ±164.84	11.5179	.0000
Systolic BP(mmHg)	149.81 ± 14.72	77.32 ± 6.73	9.6592	.0000
Diastolic BP(mmHg)	97.72 ± 10.07	116.29 ± 5.16	8.4091	.0000

Table 3: Hypertensive non smokers versus control

Variables	Hypertensive Non smokers	Control	Student's t (Critical t= 1.9960)	P value
TBARS(nmol/ml)	3.62 ± 0.81	1.98 ± 0.47	8.8065	.0000
SOD(U/gHb)	1198.47 ± 125.19	1491.04 ± 164.83	8.1750	.0000
Systolic BP(mmHg)	142.15 ± 11.34	116.29 ± 5.16	5.4925	.0000
Diastolic BP(mmHg)	94.19 ± 13.61	77.32 ± 6.73	9.3959	.0000

Table 4: Hypertensive smokers versus non hypertensive smokers

Variables	Hypertensive smokers	Hypertensive Non smokers	Student's t (Critical t= 1.9897)	P value
TBARS(nmol/ml)	4.46 ± 1.13	3.62 ± 0.81	3.9448	.0002
SOD(U/gHb)	931.66 ± 188.12	1198.47 ± 125.19	7.7451	.0000
Systolic BP(mmHg)	149.81 ± 14.72	142.15 ± 11.34	2.7040	.0009
Diastolic BP(mmHg)	97.72 ± 10.07	94.19 ± 13.61	2.6790	.0083

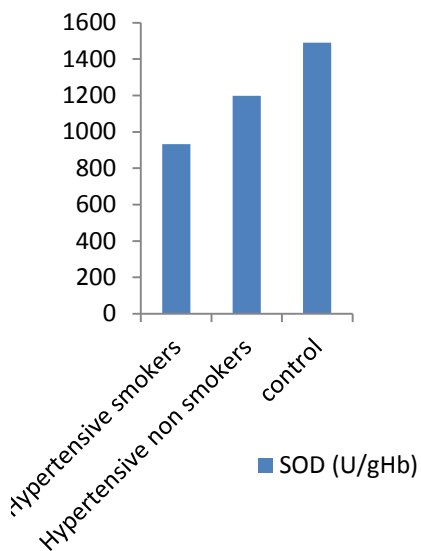


Figure I: Erythrocyte SOD activity in all groups

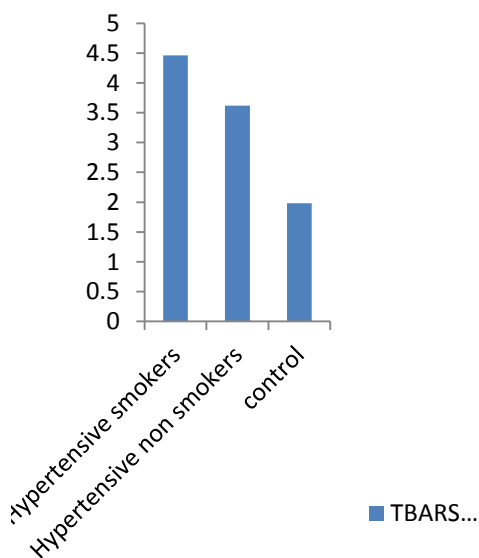


Figure II: TBARS levels in all groups

Table 1: Age, sex, population, mean ± SD of parameters estimated (TBARS, SOD, systolic and diastolic blood pressure).

Table 2, Figure I and II: Statistical comparison of values of all parameters between hypertensive smokers and control. There were statistically significant variations ($P < 0.0001$) when all parameters in hypertensive smokers were compared with that of normal non smokers.

Table 3, Figure I and II: Statistical comparison of values of all parameters between hypertensive non smokers and control. There were statistically significant variations when all parameters in hypertensive non smokers were compared with that of normal non smokers.

Table 4, Figure I and II: Statistical comparison of values of all parameters between hypertensive smokers and hypertensive non smokers. All values were found to be statistically significant when all parameters in hypertensive smokers were compared with that of hypertensive non smokers.

DISCUSSION

The pathophysiology of hypertension involves an increased peripheral resistance, resulting predominantly from functional, structural, and mechanical alterations at the level of small-resistance arteries (Virdiset al., 2011). These alterations could include an impaired endothelial function, vascular remodeling secondary to an increased cell growth, cell migration, and to a lesser extent, vascular inflammation resulting in the narrowing of the lumen (Schiffrin, 1992; Mulvany et al., 1996). Smoking has been described as a cocktail of danger by many researchers (Navas-acien et al., 2004; Torikai et al., 2004; Proctor, 2012), contaminating the smoker with so many harmful substances that has carcinogenic and other toxic dimensions on the human physiology. The systolic blood pressure is that due to the pumping effect of the heart when it beats, while the diastolic blood pressure is the pressure in the arteries when the heart rests between beats and, is therefore a reflection of the reaction of the blood vessels to the flow of blood. In this research there was a statistically significant difference when both the systolic and the diastolic blood pressures in hypertensive smokers were compared with that of non smokers. Similarly, both the systolic and diastolic blood pressure was significantly higher in hypertensive nonsmokers. This research also compared values of hypertensive smokers with that of hypertensive non smokers. Thus, both the systolic and the diastolic blood pressure in hypertensive smokers were significantly higher than that seen in non smoking hypertensives, giving an indication that the use and misuse of tobacco products has significant effects on the pumping force of the heart and also on the reaction of the arteries to the flow of blood. Though this findings links hypertension and smoking, it begs for further probe, as hypertension is a clinical manifestation of molecular and cellular interactions within the vasculature (Schiffrin, 1992; Mulvany et al., 1996), to which smoking as a lifestyle is a likely contributing factor (Navas-acien, 2004). TBARS was measured to reflect the extent of lipid peroxidation. Its inclusion in this research was predicated on the fact that any substance that will promote the pathogenesis of hypertension may do so through arterogenesis, fatty streak

atherosclerosis) formation being a sequel of lipid peroxidation (Vogiatzi et al., 2009). Lipid peroxidation was found to be significantly higher when both hypertensive smokers and non smoking hypertensives (Tables 2 and 3, Fig II) were compared with normal non smokers. Lipid peroxidation was also significantly increased in hypertensive smokers when compared with hypertensive non smokers (Table 4, Fig. II), giving an indication that smoking exacerbates lipid peroxidation in both hypertensive and normal smokers. Superoxide dismutase (SOD) is an antioxidant enzyme. Among the known antioxidant proteins, (SOD) is thought to play a central role because of its ability to scavenge superoxide anions, the primary ROS generated from molecular oxygen in cells (Fridovich, 1995), that it has been thought of as the body's firstline of defense against oxidative damage (Recklies et al., 2000). In this research, there is a significant reduction in SOD activity when both smoking and non smoking hypertensives were compared with non smoking normotensives (Tables 2 and 3, Fig. 1), making it evident that hypertension is associated with reduced antioxidative SOD activity. Similarly, SOD activity was significantly lower in smoking hypertensives when compared with non smoking hypertensives (Table 4, Fig. 1) indicating that smoking is associated with the depletion of SOD activity and the reason could not be farfetched; Smokers are exposed to so many harmful substances, for example, cigarette smokers are contaminated through cigarette with nicotine and also heavy metals such as lead, cadmium, arsenic and mercury. Halimi et al., (1998) stated that smoking is associated with an acute increase in arterial pressure due to systemic vasoconstriction, decreased skin and coronary blood flow, nicotine being associated with the cardiovascular effect. As regards the deleterious effects of the heavy metals, SOD is a metalloenzyme, upon contamination with any of the heavy metals mentioned above, they are likely to replace Cu, Mn and Zn as the metal constituents of SOD (Huang et al., 2006). When this happens the enzyme activity is diminished, therefore the level of the superoxide, which should have been scavenged increases, leading to, among other conditions, accelerated lipid peroxidation..When anti oxidant levels decrease, the superoxide ion accumulates in the system, leading to the development and progression of a variety of cardiovascular diseases (Touyz et al., 2003; Navas acien et al., 2004; Touyz and Schifrin., 2004). In fact this is the summary as explained in the works of friendling et al., (2000)

and landmesser and Harrison (2001), of the ways by which ROS perform their pathophysiological role in cardiovascular dysfunction associated with several clinical conditions.As HTN is a major independent risk factor for coronary artery disease, stroke, and kidneyfailure(Tabassum and Ahmad, 2011), if there is any truth in the saying in the work of Tabassum and Ahmad, 2011 that“each increase of 20 mmHg in systolic BP and 10 mmHg in diastolic BP, over therange of 115/75 to 185/115 mmHg, doubles the risk of a fatal coronary event”, then the significant increase in both the systolic and diastolic BP of the hypertensive smoker over thenon smoking hypertensive subjects gives the indication that smoking as a lifestyle aggravates an already existing hypertensive state. In fact, there is a possibility that hypertensive smokers are being undercared for, if given the same set of treatments as the non smoker.

CONCLUSION

This research found that smoking exposes these subjects under examination to substances which directly or otherwise increase blood pressure. Thus,increased lipid peroxidation and reduced antioxidant enzyme activity as seen in hypertensives when compared with control and when hypertensive smokers was compared with hypertensive non smokers proves further that reduced antioxidant status, smoking and, elevation of blood pressure, are interrelated. It appears smoking is a contributing factor to the generation of reactive oxygen species, hence the classical peculiarities of hypertension.

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