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Iron Overload Reduces IL10, GMCSF and increases IL5 and TGF Levels in Nigerian Hypertensives

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Abstract

Hypertension is a leading cause of mortality and contributes to disease burden worldwide. The role of cytokines as inflammatory modulators and trace metals in systemic diseases is well documented. However, the interplay of trace elements with cytokines in hypertension has not been fully elucidated especially in this environment. This work investigated the interplay of some trace metals (Fe, Zn, Pb and Cd) with cytokines (IL2, IL4, IL5, IL6, IL10, IL12, IL13, IL17, IFN, TNF, GMCSF and TGF) in hypertension. Fourteen clinically diagnosed hypertensives (mean BP 139/88) attending the University College Hospital, ten non-hypertensives (mean BP 114/72) were also chosen as controls (age range: 40-60years). Blood lead (Pb), Cadmium (Cd), Iron (Fe) and plasma Zinc (Zn) were determined in all subjects using atomic absorption spectrophotometry while ELISA technique was used to determine the levels of cytokines. Levels of toxic trace metals obtained in the normotensive and hypertensive subjects were within acceptable limits, however, level of Fe (mean 60.2µg/dl), (CI-42.4-78.0) and Zn (mean 7.6µg/dl), (CI-5.9-9.4) obtained in hypertensive subjects were significantly higher than in normotensive subjects [(mean 49.8µg/dl) and (4.9µg/dl)] for Fe and Zn respectively ($P<0.05$). Proliferation of proinflammatory cytokines was not statistically different in both control and hypertensive subjects except for IL5 and TGF which were raised ($p<0.05$) while IL10 and GMCSF were reduced, ($P<0.05$, respectively) in hypertensive relative to normotensive subjects. The interplay of the observed iron overload with the cytokines and the possible pathological implication of this in the development and progression of hypertension are discussed in this work.

Keywords: Hypertensives, Iron overload, Trace metals, Proinflammatory cytokines,

Introduction

Cardiovascular disease (CVD) (also called heart disease) is a class of diseases that involve the heart or blood vessels (arteries, capillaries and veins). CVD refers to any disease that affects the cardiovascular system, principally cardiac disease, vascular diseases (hypertension) of the brain and kidney and peripheral arterial disease (Bridget *et al.,* 2010). Cardiovascular diseases are common in this modern age; it is the leading cause of mortality and a primary contributor to the burden of disease worldwide (Lopez *et al.,* 2006). The causes of cardiovascular disease are diverse but atherosclerosis is the most common. There are different cardiovascular diseases, some may be present from early age or from birth such as rare malformations of the structure of heart, and others take a life time to acquire such as the Angina Pectoris (Bukhari *et al.*,2005). Epidemiology suggests a number of risk factors for heart disease: age, gender, high blood pressure, high serum cholesterol levels, tobacco smoking, excessive alcohol consumption, family history, obesity, lack of physical activity, psychosocial factors, diabetes mellitus and environmental pollutants (Bridget *et al*., 2010). Although cardiovascular disease usually affects older adults, the antecedents of cardiovascular disease, notably atherosclerosis, begin in early life which develops over many years and is usually advanced by the time symptoms occur. Inflammation has been documented as one of major weapons needed for the progression of cardiovascular disease (McGill *et al*., 2008). Hypertension remains one of the hallmarks of cardiovascular diseases.

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Hypertension (HTN or HT), also known as high blood pressure or arterial hypertension, is a chronic medical condition in which the blood pressure in the arteries is elevated. Hypertension is classified as either primary (essential) hypertension or secondary hypertension and is diagnosed if the blood pressure is persistently at or above 140/90 millimeters mercury (mmHg) for most adults; (James *et al.*, 2013)

About 90–95% of cases are categorized as primary hypertension, defined as high blood pressure with no obvious underlying cause. The remaining 5–10% of cases are categorized as secondary hypertension, defined as hypertension due to an identifiable cause, such as chronic kidney disease, narrowing of the aorta or kidney arteries, or an endocrine disorder such as excess aldosterone, cortisol, or catecholamines.

The exact pathophysiology of hypertension remains an issue of debate; however, increased peripheral resistance in established hypertension stands out as one of the hallmarks of this condition. Many mechanisms have been proposed to account for the rise in peripheral resistance in hypertension while many factors have been implicated. Amongst these factors are endothelial dysfunction and vascular inflammation. Establishment of involvement of inflammation in the development and progression of hypertension has focused attention on immune system chemicals (Interleukins and interferon); hence the focus of this work is on the interplay of these chemical inflammatory signals in the aetiogenesis of hypertension.

Trace elements are crucial for maintaining human health, as well as for preventing several health problems. Alteration of normal homeostasis of trace elements may adversely affect biological processes leading to many disease processes [Orshal and Khalil, 2004]. So many of these trace elements have been identified, amongst them are Zinc (Zn), Iron (Fe), Cadmium (Cd) and Lead (Pb). Zn plays a substantial role in metabolic processes; it is involved in more than 300 different enzymes [Zhang *et al.*, 2003]. Zn acts as an intracellular signalling molecule which is able to communicate between cells by converting extracellular stimuli to intracellular signals and controlling intracellular actions. Normal homeostasis of Zn is regulated by the harmonized actions of Zn transporters such as Zinc-and Iron-related Protein (Zip) which controls Zn influx and efflux and thus controls the concentration of Zn inside and outside the cell [Saffar *et al.*, 2003; Siwik and Colucci, 2004]. Thus, alteration of Zn homeostasis and dysfunction in the signalling function of Zn may cause pathogenesis of several diseases [Ruiz-Ortega *et al.*, 2002; Sanz-Rosa *et al.*, 2005].

Like Zn, Fe is also an essential microelement involved in almost all oxidative processes of the body. It is a micro element that is essentially derived from diet. Its absorption, transportation and uptake by the body is strictly regulated by a number of processes; this is because its deficiency is as dangerous as its overload. Whilst iron is essential for cellular metabolism and aerobic respiration, its deficiency results in anaemia. However, cellular iron overload leads to toxicity and cell death via free radical formation and lipid peroxidation and thus iron homeostasis requires tight regulation (Chiang et al, 2006). Unlike in Europe where haemochromatosis is more of an inherited disease, in Sub-Sahara Africa it is usually derived from excessive ingestion. However, problem associated with iron overload is so multi-faceted and as complex as its regulatory mechanism in the human system.

Environmental toxicants, including lead and other metals, are potential contributors to cardiovascular disease (Bhatnagar 2006; Weinhold 2004). Lead is a prime toxicant and environmental pollutant that affects virtually every system in the body. Like other toxic metals, lead damages cellular material and alters cellular genetics. Lead (Pb) has no nutritional function but is an ubiquitous environmental toxin that is capable of causing numerous acute and chronic illnesses (Tong *et al.*, 2000). Bone serves as the principal repository of this element in the body and gradual release of lead from the bone serves as a persistent source of toxicity long after cessation of external exposure. The kidney is the principal route of lead excretion. However, soil contamination is a persistent source of lead exposure in the industrial societies. A number of reports have demonstrated a link between lead exposure and development of hypertension (HTN) and cardiovascular disease. In 2008, Vaziri and his group reported possible connection between lead exposure and other cardiovascular disorders including ischaemic coronary heart disease, cerebrovascular accidents, and peripheral vascular disease (Vaziri, 2008). Several reviews and meta analyses combining data from more than 30 studies have examined the evidence relating blood lead to blood pressure or hypertension (Hertz-Picciotto and Croft 1993; Nawrot *et al.*, 2002;

Schwartz 1995; Sharp *et al.,* 1987; Staessen *et al.,* 1994,1995; U.S. Environmental Protection Agency (U.S. EPA) 2006). The preponderance of opinion by these reviewers was that there was a positive association between blood lead levels and blood pressure. The cardiovascular effects of lead, however, are not limited to increased blood pressure and hypertension. Lead exposure has also been associated with an increased incidence of clinical cardiovascular end points such as coronary heart disease, stroke, and peripheral arterial disease (Lustberg and Silbergeld 2002; Menke *et al*., 2006; Navas-Acien *et al*., 2004; Schober *et al*., 2006), and with other cardiovascular function abnormalities such as left ventricular hypertrophy and alterations in cardiac rhythm (Cheng *et al*., 1998; Schwartz 1991).

Environmental exposure to cadmium was associated with significantly increased stroke and heart failure prevalence. Cadmium exposure may increase these important manifestations of cardiovascular disease (Peters et al 2010). According to Agency for Toxic Substances and Disease Registry (ATSDR) (1999) and International Agency for Research in Cancer (IARC) (1994), Cadmium is a non-essential metal widely distributed in the environment. It is a by-product from mining, smelting and refining of zinc, lead and copper ores; cadmium production and use have substantially increased, particularly in nickel-cadmium batteries, fertilizers, coatings and plastic stabilizers. Experimental evidence also suggests that cadmium could directly induce atherosclerosis initiation and progression (Valko *et al.,* 2005; Messner *et al.,* 2009). Cadmium causes endothelial dysfunction in vitro, and accelerates atherosclerotic plaque formation *in vivo*. Several mechanisms have been suggested to explain the role of cadmium in promoting atherosclerosis. First, cadmium, a divalent cation binds sulphydryl group-containing enzymes and this could indirectly increase reactive oxygen species formation. It also interferes with anti-oxidative stress responses by binding metallothionein, a low molecular weight protein that regulates zinc homeostasis and acts as a free radical scavenger (Valko *et al*., 2009; Maret *et al*., 2009). Secondly, cadmium may inhibit the cell cycle by altering cell signalling pathways and induce an atypical form of apoptosis involving necrotic rupture of the endothelial cell plasma membrane, with subsequent attraction and activation of macrophages. Thirdly, cadmium may partly contribute to atherosclerosis formation through vasopressor mechanisms such as direct vasoconstrictor action, inhibition of vasodilator substances such as nitric oxide, or activation of the sympathetic nervous system (Varoni *et al*., 2003; Bilgen ., 2003). In the kidney, cadmium may induce salt retention and volume overload, which may produce hypertension. It is also possible that cadmium, a well-known nephrotoxicant in occupational settings, contributes to systemic hypertension and atherosclerosis partly by producing injury to the kidney, a key organ in blood pressure regulation (Nordberg *et al*., 2007; Satarug, *et al*., 2010; Jarup *et al*., 2009).

Many chronic diseases have been associated with exposure to toxic trace metals, however, identification of early biomarkers of these diseases remain a scientific challenge even in the developed world. A number of reports from National Health and Nutrition Examination Survey (NHANES,1999-2008) found associations of cadmium biomarkers with different cardiovascular related end-points including blood pressure levels (Whittemore *et al*., 1991; Tellez-Plaza ., 2008), kidney disease, diabetes and peripheral arterial disease (Navas-Acien *et al*., 2004,2005). Houtman et al (1993) and Voors et al (1977) also found associations between tissue cadmium levels and atherosclerotic lesions in their different autopsy studies. Although several markers have been proposed with their various advantages and disadvantages, the interaction of these trace elements with various inflammatory markers such as cytokines and chemokines could facilitate the diagnosis and management of some of these chronic diseases.

Cytokines are proteins that are produced by cells; they serve as molecular messengers between cells and interact with cells of the immune system in order to regulate the body's response to disease and infection. The role of cytokines in the pathogenesis of cardiovascular disease is increasingly evident since the identification of immune/inflammatory mechanisms in atherosclerosis and heart failure. Inflammation is a complex of defence mechanisms reacting to entry of harmful organism or cells in order to eliminate or dilute the agent, repair damaged cells or tissue and restore homeostasis. It plays an important role in the aetiology of many chronic diseases including coronary heart disease, myocardial infarction, angina pectoris and hypertension. The pathophysiology of cytokines include its depressant effect on myocardial function due to the generation of nitric oxide (NO) as well as the biochemical effects of cytokine-stimulated arachidonic acid metabolites on cardiomyocytes. While the cardiovascular effects of lead and cadmium are not limited to increased blood pressure and hypertension; it is worth examining the relationship between these trace elements (Zn, Fe, Cd and Pb) and production of

cytokines ((IL2, IL4, IL5, IL6, IL10, IL12, IL13, IL17, IFN, TNF, GMCSF and TGF) which are known inflammatory makers in the development and progression of hypertension in this environment (Mendis et al 2011).

Materials and Methods

Twenty (20) subjects aged (40 - 60) years, male and female clinically diagnosed and managed for hypertension (mean blood pressure 139/88mmHg) at the medical out-patient department of UCH, Ibadan were selected as test subjects. The control group (20 people) consisted of males and females hospital staff and others. They were of the same age range (40 -60), normotensive (mean blood pressure 114/72mmHg) and with no history of other cardiovascular diseases or any occupational exposure to any of the trace metals (or their agonists) under investigation. Ethical approval for this work was obtained from the UI/UCH ethical Committee.

Blood samples (10ml) were collected from subjects with all necessary precautions to ensure reliability of results (blood for the trace metals and serum for the cytokines)

Trace metals analysis

Pb, Cd, Zn and Fe were determined using Atomic Absorption Spectrophotometry (AAS) based on modified method of Hessel (1968). The blood samples were wet digested using nitric acid and perchloric acid mixture (3:2) while analysis was done on AAS Buck equipment model 210VGP (manufactured by Buck Scientific Corporation, USA) using air/acetylene mixture. The detection limits of the equipment are 0.05µg/dl, 0.5µg/dl, 0.003µg/dl and 0.01µg/dl for Pb, Fe, Zn and Cd respectively. All procedures were carried out based on standard laboratory practice and control.

Serum Cytokine analysis

Serum levels of 12 cytokines IL-2, IL-4, IL 5, IL-6, IL 10, IL 12, IL 13, IL 17A, IFN-γ, TNF-α, GM CSF and TGF-β) were determined using Multi-analyte ELISArray Kits based on the method described by the manufacturer (from SA Biosciences, USA). The average absorbance of each sample was determined from a standard curve of absorbance versus corresponding cytokine value of the manufacturers' standard solution.

Data Analysis

Data were analyzed using SPSS and the values presented as mean with confidence interval. The differences in values were compared using ANOVA and Student t-test. P-values at ≤ 0.05 were taken as statistically significant.

RESULTS:

Median trace element concentrations with their inter quartile range are 49.8mg/dl (35.5 – 64.4), and 60.2mg/dl (42.4 – 78.0) for Fe and 4.9 µg/dl /dl (3.4 – 6.5) and 7.6 µg/dl /dl (5.9 – 9.4) for Zn in normotensives and hypertensives respectively. The values obtained for Fe and Zn in hypertensives were significantly higher than in normotensives ($P \leq 0.05$). Specifically, iron overload was observed in hypertensive subjects relative to the control (Median values of 60.2µg/dl and 49.8µg/dl respectively). Serum Zn level was also upregulated in hypertensives (mean 7.6 µg/dl) in comparison with the control (mean 4.9 µg/dl). The mean levels of toxic metals (Pb and Cd) were within acceptable limits in both control and hypertensive subjects (less than 5µg/dl respectively), the differences were statistically nonsignificant ($p>0.05$). (Tables 1)

The serum cytokines determined could broadly be classified as; pro inflammatory and the anti inflammatory cytokines. Although, there are degrees of overlap in functions of some cytokines, however, cytokines known to mediate chronic inflammatory processes can be divided into those initiate humoral inflammation, such as IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-13, and transforming growth factor-b (TGF-b), and those initiating cellular inflammation such as IL-1, IL-2, IL-3, IL-4, IL-7, IL-9, IL-12, interferons (IFNs), IFN-g inducing factor (IGIF), TGF-β, and TNF-α and -β. Of all the proinflammatory cytokines determined (IL2, IL4, IL5, IL6, IL12 and IL13), only IL5 was found to be upregulated in hypertensive subjects relative to what was obtained in the normotensive subjects, ($p<0.05$); the rest were not significantly different in both the hypertensive and normotensive subjects ($P \geq 0.05$). In contrast to this, IL 10 an anti inflammatory cytokine was down regulated in hypertensive subjects relative to the controls in this study. Also, the chemokines, TGF-β was found to be markedly upregulated in hypertensive subjects ($P \leq 0.05$) while there was a slight down regulation of GM-CSF in hypertensive subjects relative to the controls in this study ($P \leq 0.05$) (Tables 1).

A comparative graphical representation of the trace metals level is also presented in figures 1 and 2 below.

TABLE 1: Iron and Zinc Levels in Normotensive and Hypertensive Subjects

Parameters	Normotensive Subjects Mean (95% Confidence Interval)	Hypertensive Subjects Mean (95% Confidence Interval)	P-Value
Fe (mg/dl)	49.8 (35.5 – 64.4)	60.2 (42.4 – 78.0)	0.011*
Zn (µg/dl)	4.9 (3.4 – 6.5)	7.6 (5.9 – 9.4)	0.000*

* Significance level is at 0.05

Table 2: Summary of Cytokine levels in Normotensive and Hypertensive Subjects

Parameters	Normotensive Subjects Mean (95% Confidence Interval)	Hypertensive Subjects Mean (95% Confidence Interval)	P-Value
IL2	0.28 (0.27 – 0.29)	0.27 (0.26 – 0.28)	0.173
IL4	0.28 (0.27 – 0.29)	0.27 (0.26 – 0.28)	0.068
IL5	0.29 (0.27 – 0.30)	0.32 (0.30 – 0.33)	0.006*
IL6	0.28 (0.27 – 0.29)	0.27 (0.27 – 0.28)	0.187
IL10	0.27 (0.26 – 0.28)	0.22 (0.20 – 0.24)	0.000*
IL12	0.27 (0.26 – 0.28)	0.26 (0.26 – 0.27)	0.262
IL13	0.27 (0.26 – 0.28)	0.27 (0.26 – 0.27)	0.080
IL17	0.28 (0.27 – 0.28)	0.27 (0.26 – 0.28)	0.387
IFN	0.27 (0.26 – 0.28)	0.27 (0.27 – 0.28)	0.175
TNF	0.27 (0.27 – 0.28)	0.28 (0.27 – 0.28)	0.355
GMCSF	0.28 (0.28 – 0.29)	0.28 (0.27 – 0.28)	0.013*
TGF	0.74 (0.51 – 0.97)	1.07 (0.90 – 1.23)	0.006*

*Significance level is at 0.05

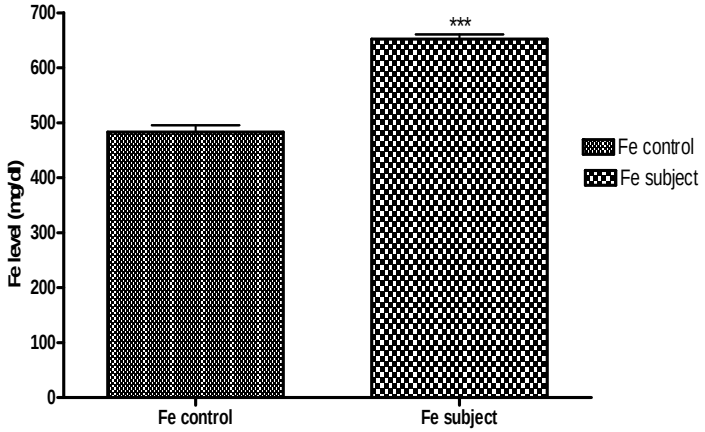

Figure 1: Comparative graph of serum Fe levels in Normotensive and Hypertensive Subjects

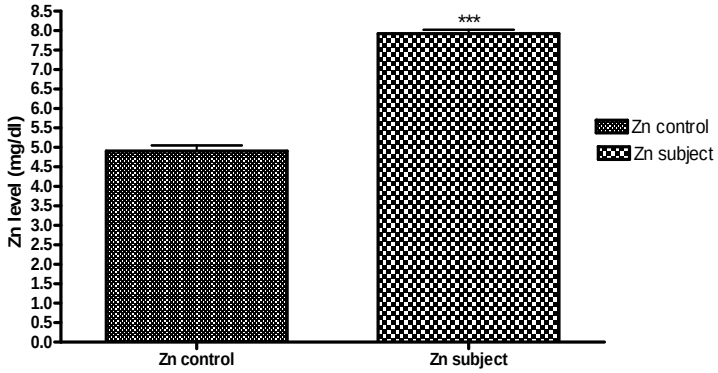

Figure 2: Comparative graph of Serum Zn in Normotensive and Hypertensive Subjects

Table 3: Summary of Trace Metals and Cytokine Levels in Normotensive and Hypertensive Subjects

Parameters	**Normotensive Subjects Mean (95% Confidence Interval)**	**Hypertensive Subjects Mean (95% Confidence Interval)**	**P-Value**
Fe (mg/dl)	49.8 (35.5 – 64.4)	60.2 (42.4 – 78.0)	0.011*
Zn (µg/dl)	4.9 (3.4 – 6.5)	7.6 (5.9 – 9.4)	0.000*
IL2	0.28 (0.27 – 0.29)	0.27 (0.26 – 0.28)	0.173
IL4	0.28 (0.27 – 0.29)	0.27 (0.26 – 0.28)	0.068
IL5	0.29 (0.27 – 0.30)	0.32 (0.30 – 0.33)	0.006*
IL6	0.28 (0.27 – 0.29)	0.27 (0.27 – 0.28)	0.187
IL10	0.27 (0.26 – 0.28)	0.22 (0.20 – 0.24)	0.000*
IL12	0.27 (0.26 – 0.28)	0.26 (0.26 – 0.27)	0.262
IL13	0.27 (0.26 – 0.28)	0.27 (0.26 – 0.27)	0.080
IL17	0.28 (0.27 – 0.28)	0.27 (0.26 – 0.28)	0.387
IFN	0.27 (0.26 – 0.28)	0.27 (0.27 – 0.28)	0.175
TNF	0.27 (0.27 – 0.28)	0.28 (0.27 – 0.28)	0.355
GMCSF	0.28 (0.28 – 0.29)	0.28 (0.27 – 0.28)	0.013*
TGF	0.74 (0.51 – 0.97)	1.07 (0.90 – 1.23)	0.006*

*Significance level is at 0.05

Discussion:

Hypertension is one of the leading causes of death worldwide; however, in spite of the volume of scientific knowledge on this disease, the actual pathophysiology especially as it progresses remain uncertain. As earlier stated, the focus of this study was to determine the role of some trace metals on the generation of cytokines and their influence on the pathogenesis of hypertension

From this work, serum iron and zinc levels were raised in most of the hypertensive subjects studied while levels of cadmium and lead were negligible or non significant. Amongst issues raised is the source of the iron and zinc overload. In many studies, iron overload or primary haemochromatosis has been established as a genetic disorder caused by mutation of the gene controlling the absorption of Fe especially in homozygous substitution of tyrosine for cysteine at position 282 (C282Y) in the HFE protein that regulate Fe uptake in the intestine. This genetic aberration is known to be common in Europe (Katrina et al, 2008). However, in Africans, iron overload is essentially due to increase dietary ingestion of this metal which originally was traced to the "Bantus", a tribe in East Africa (Kasvosve *et al.,* 2008). Although possibility of genetic disposition (different from the genetic loci that obtains in Europeans) has also been discussed, dietary sources have largely been implicated as the major source of primary haemochromatosis amongst Africans. Though information on dietary regimens of participants in this study were not documented, iron overload observed in hypertensive subjects in this study might have resulted after a long term consumption of iron rich food or could have been a result of a different genetic disposition of this hypertensive stops increased absorption of iron in their alimentary system (Moyo *et al.*, 1998). Unfortunately, demographic data that could have helped in establishing either were not collected in this study. Possibility of an inadvertent chronic exposure to iron from dietary sources is heightened by the indiscriminate use of food supplement drugs most of which contain a high level of iron. The practice of indiscriminate administration of these supplements has almost become a vogue amongst the middle class/age community in this environment. The cumulative effect of chronic exposure to these non-prescribed drugs could therefore be a veritable source of iron resulting in the observed overload in the subjects. However, it is pertinent that the different genetic disposition reported by Moyo et al (1998) and Kasvosve et al (2008) in the genetic disorder of iron absorption in Africans notwithstanding, there is a general lack of data on the basis of haemochromatosis in African hypertensives at least in this environment. Rather, most works on this disorder has been on sickle cell, thalasaemia and other haematological abnormalities. Thus, it may be too preposterous to rule out hereditary heamochromatosis (HH) which is an autosomal-recessive disorder of iron metabolism as a strong basis of the observed iron overload. Primary haemochromatosis has been associated with hepatic diseases (hepatoma and cirrhosis) resulting in high premature mortality (Niederau, 1985), heart failure has equally been

strongly implicated in patients with HH or secondary haemochromatosis (Aldouri et al, 1990; Gabutti and Borgna-Pitnatti, 1994). Although this genetic abnormality might be rare in this part of the world, we are not aware of studies establishing the prevalence of HH and its link with various diseases of iron overload (especially hypertension) in this environment. The need to map out the link between this condition and the myriad of unresolved hypertensives in this environment cannot therefore be overlooked.

Several works have reported on the pathological consequences of iron overload which essentially involve iron-driven oxidation reactions which though may be gradual; usually result in organ damage/failure. Excess Fe load is as dangerous as its deficiency because Fe, based on Fenton chemistry, generates singlet oxygen and other ROS which could have compromised the antioxidant potentials of these subjects. The liver, heart and pancreas have been reported to be the most vulnerable organs for this damage, this is largely attributed to the high rate of production of superoxide (O_2^-) and H_2O_2 largely derived from mitochondrial activity (Chance, 1979). Chronic iron overload has also been reported to present clinically with a dysfunction in systolic pressure (Liu and Olivier, 1994). Thus, characteristically, the systolic pressure which occurs when the left ventricle is most contracted may be linked to either over polarization or over stretch of the ventricular muscles. It can thus be said that the mean systolic BP of 138mmHg observed in the group of hypertensive subjects in this study relative to that of the controls (114mmHg) also corroborated the presence of insidious organ damage known to be peculiar to heart and liver in iron overload (Chiang et al, 2006).

Zn is an ubiquitous and essential metal which is associated with many metabolic functions of the body. Although there are no known direct toxic effects of excess Zn; its toxicity has been related directly to its ability to regulate the absorption of copper in the intestine and the protective action against toxicities of lead and cadmium in the body. Hence, increased plasma zinc level observed in the hypertensive subjects relative to the controls in this study may be the protective effect against the toxicity of lead and cadmium or may be related to the plasma level of copper in these subjects. Unfortunately, plasma copper level was not determined in this study. Prasad (2000) reported skewing effect of some metals on the immunological system of the body where increased Zn level was found to have skewed production of Th1 to Th2 due to exposure of some subjects to Pb and Hg. Thus, the compensatory protective measure against the levels of the toxic trace metals (Pb and Cd) due to increase in Zn level observed in this study may be similar to what was reported by Prassad in his study.

Results of cytokines and chemokines determined in this study showed some relationship to the skewing of immunological parameters by the presence of some of the trace metals. In iron overload, although GSH and other cellular reducing species can act as antioxidant, this same reducing agent can have pro-oxidant effects when acting as part of a metal-catalyzed redox couple (Scott and Taton, 1995). The cumulative effects of the imbalance in oxidant/antioxidant ratio could possibly be responsible for the shift in the immune balance of subjects with hypertension. Hence, the observed reduction in the level of IL10 (an anti-inflammatory cytokine) might be a direct consequence of this. The observed shift from Th1supportive cytokines (IL2 etc) to Th2 (as depicted by increase in TGF) could be due to either the presence of intracellular pathogen (which may be secondary or possibly opportunistic) due to the suppressed or depressed immunity. This could possibly be the basis of the raised TGF levels (a stimulatory chemokine) observed in this hypertensive subjects.

Interestingly, a slight up regulation of IL5 was observed in the hypertensive subjects in this study. IL5 is a known activator of eosinophilia which is largely associated with immunity against parasites; the concurrent presence of parasitic infestation in the subjects could possibly explain the slight increase in IL5 observed in the hypertensive subjects. Parasitic infestation especially amongst the populace is not uncommon in sub-Sahara Africa; hence a concurrent presence of parasitaemia as indicated by the down regulated IL5 could have also contributed to the dampening of the immune system thus compromising the overall defence mechanism of the body.

As earlier stated, capillary resistance is one of the basis of progression of hypertension in iron overload. Nobukazu and his team (2002) proposed that iron overload promotes cardiac fibrosis and neointima formation by augmenting Angiotensin II in the development of arthrosclerosis in experimental animals. Although the reactive oxygen species that are directly responsible for the formation of fibrotic regions in the heart are yet to be identified, generation of hydroxyl radicals through these reactions may play important roles in the formation of cardiac fibrosis and progression of hypertension; the pathophysiology may be similar to those proposed for other organs

susceptible to iron overload [Svegliati-Baroni et al, (2001); Bruck et al, (2001)] .

Conclusion: The observed interplay between trace metals (essential and toxic) and the cytokines could possibly be a case of haemochromatosis producing imbalance in cytokine levels in the system; the pro inflammatory effect of which auguments the development of fibrotic changes in the heart muscles. This coupled with the possible presence of parasitaemia may dampen the immune capacity of the body thus facilitating progression of hypertension. The possibility of secondary complications underscores the need to guard against heart failure hypertensive subjects since an increased risk of heart failure and progression into diabetes has been found to be high (Aldouri et al, 1990; Gabutti and Borgna-Pitnatti, 1994) in subjects with haemochromatosis secondary to imbalance in cytokine levels.

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Comments on Presentation of Results

Presentation of result, I suggest, should be ordered in a logical sequence. Authors may consider the following order:

1. **Trace metals**
2. **Serum cytokines**
3. **Trace metals and cytokines levels in hypertensive and normotensive**

Comments on Discussion

1. **The Discussion of results is scanty**
2. **I suggest that the Discussion should follow the sequence of the Results as presented:**
   - **(i) Distribution (levels) of trace metals in the study group**
   - **(ii) Metals implicated in hypertension**
   - **(iii) Serum cytokines and hypertension**
   - **(iv) Comparative analysis of Results of other authors and this work**
   - **(v) Etc**
3. **Conclusion**

Reviewer's Submission

The work is novel and publishable but not in this present state/format. It requires some modification and editorial work (proof-reading).

The Editor should look at the references to ascertain conformity with the journal format.

Thank you

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