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SERUM MARKERS OF IRON STATUS AND β2 MICROGLOBULIN IN PATIENTS WITH INCIDENT HYPERTENSION

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ABSTRACT

Prior studies have associated B_2 microglobulin with cardiovascular disease. The cross-sectional study, aimed at determining the level of B_2microglobulin and markers of iron status in patients with incident hypertension, was conducted in the month of July 2021 and all eligible subjects who filled the questionnaire and gave a written informed consent for the study period were enrolled. A total of 150 subjects (comprising of 50 male hypertensive patient, 50 female hypertensive patients and 50 normotensive subjects were recruited randomly for the study. Their blood pressures, determined. Blood samples collected were also used in the assay of iron, ferritin, total iron binding capacity and B_2microglobulin using standard laboratory procedures.

All data obtained in the study were analyzed using student t-test (spss.21) and Pearson correlation coefficient. The level of significance was set at $p < 0.05$. The mean value of systolic blood pressure was significantly increased (p=0.006) in patients with incident hypertension (120.81±5.34) mmHg when compared to normotensives (113.30±5.83) mmHg. The mean value of diastolic blood pressure was significantly increased (p=0.008) in patients with incident hypertension (80.82±4.53) mmHg when compared to normotensives (75.20±4.05) mmHg. B_2M was significantly increased (p=0.007) in patients with incident hypertension (3.08±0.49) ng/ml compared to normotensives (2.50±0.27) ng/ml. Haemoglobin and Iron concentrations were significantly reduced (p=0.006), (p=0.070) in patients with incident hypertension (10.83±0.78) g/dl, (105.28±23.19)µg/dl when compared to normotensives (12.941±0.86)g/dl, (122.78±17.94)µg/dl respectively. The mean values of ferritin and TIBC were significantly increased (p=0.381), (p=0..562) in patients with incident hypertension (120.22±24.35)ng/ml, (303.79±14.37)µg/dl when compared to normotensives (110.37±26.04)µg/dl, (299.68±17.54)µg/dl. There was a non significant negative correlation of B_2M with Hb, iron and ferritin (r=-0.41, p=0.207; r=-0.02, p=0.963 and r=-0.28, p=0.399). There was a non significant positive correlation of B_2M with TIBC (r=-0.19 p=0.574). From the study, Incident hypertension is associated with significant increase in B_2microglobulin level, and a significant decrease in haemoglobin concentration, but there was no significant association between incident hypertension and iron status. Increase in B_2microglobulin infers inflammatory activities which may predispose victims to cardiovascular diseases.

Keywords*: serum markers, iron status, β2 microglobulin, hypertension*

INTRODUCTION

Hypertension (HTN or HT), also known as high blood pressure (HBP), is a long-term medical condition in which the blood pressure in the arteries is persistently elevated (Navar, 2010). High blood pressure usually does not cause symptoms. Long-term high blood pressure, however, is a major risk factor for coronary artery disease, stroke, heart failure, atrial fibrillation, peripheral vascular disease, vision loss, chronic kidney disease, and dementia (WHO, 2011; Obeagu *et al.,* 2018; Nnatuanya *et al.,* 2017; Nwovu *et al.,* 2018).

Blood pressure (BP) is defined as lateral pressure exerted by the blood on the walls of the blood vessels while flowing through them. Blood pressure in a blood vessel depends upon two things. Blood pressure is more in blood vessels close to the heart. Blood pressure is more in arterial system than in the venous system. This is because walls of arteries are thicker and less elastic; the walls of the veins are thinner and more elastic. Normal blood pressure is 120/80 mmHg, Systolic BP (SBP) is the maximum BP during the ventricular systole- 120 mmHg. Range: 110-130 mmHg. Diastolic BP (DBP) is the minimum pressure during the ventricular diastole. It is 80 mmHg (Lau *et al.,* 2017).

High blood pressure is classified as either primary (essential) high blood pressure or secondary high blood pressure. About 90–95% of cases are primary, defined as high blood pressure due to nonspecific lifestyle and genetic factors. Lifestyle factors that increase the risk include excess salt in the diet, excess body weight, smoking, and alcohol use. The remaining 5–10% of cases are categorized as Frequency 16–37% globally, deaths 9.4 million / 18% (2010) (Campbell *et al*., 2015). Secondary high blood pressure, defined as high blood pressure due to an identifiable cause, such as chronic kidney disease, narrowing of the kidney arteries, an endocrine disorder, or the use of birth control pills.

Blood pressure is expressed by two measurements, the systolic and diastolic pressures, which are the maximum and minimum pressures, respectively. For most adults, normal blood pressure at rest is within the range of 100–130 millimeters mercury (mmHg) systolic and 60–80 mmHg diastolic (Cornier *et al*., 2008). For most adults, high blood pressure is present if the resting

blood pressure is persistently at or above 130/90 or 140/90 mmHg. Different numbers apply to children. Ambulatory blood pressure monitoring over a 24-hour period appears more accurate than office-based blood pressure measurement.

Lifestyle changes and medications can lower blood pressure and decrease the risk of health complications. Iron is the first and the most important trace element in cellular metabolism. It occurs at many metabolic mechanisms including the modulation of the immune system and more particularly in the production of hemoglobin by the process of erythropoiesis (Lau *et al*., 2010).Ferritin is a universal intracellular protein that stores iron and releases it in a controlled fashion, while transferrins are involve in the transport of iron (Sophie *et al.,* 2014). Iron metabolism control occurs at the level of the body to maintain the stock of iron and its distribution to adequate levels, but also at the cellular level, thereby ensuring optimal biological functions (Lau *et al*., 2010).

Several studies have reported an association between serum ferritin concentration and high blood pressure and it has been suggested that disturbances of iron metabolism are part of the metabolic syndrome, which associates with raised blood pressure (Haddad *et al*., 2017).

Beta2-microglobulin (β2M) is a small molecular protein (11.8 kDa) that is secreted from all nucleated human cells with daily synthesis rates ranging from 2–4 mg/kg per day in healthy individuals. Serum β2M is metabolized almost exclusively (99%) from the kidneys and remains stable at 1–3 mg/mL (Zumrutdal, 2015). As one of the classical low-molecular-weight markers of kidney function, the serum β2M level is highly inversely associated with the glomerular fltration rate. At the same time, serum β2M concentration is often influenced by many non renal determinants, such as systolic blood pressure, gender, total cholesterol, inflammation, and smoking (Stanga, 2013). β2M is a critical component of the major histocompatibility class I (MHCI) complex heterodimer that presents intracellular antigens to cytotoxic T cells (Filiano and Kipnis, 2015) . β2M dissociates from the cell surface or releases from inside of the cell and then sheds into the blood. Te serum levels of β2M are associated with a variety of autoimmune

diseases, tumors, infectious diseases, and renal disease (Kals, 2013). However, recent studies have shown that β2M is also associated with a high risk of peripheral artery disease (PAD), cognitive dysfunction, and prevalent asymptomatic carotid atherosclerosis (Amighi, 2015). Therefore, more evidence indicates that serum β2M is not only a marker of kidney function but also has other functions in inflammatory diseases.

It was recently reported that erythropoietin—closely associated with iron or ferritin metabolism—can increase oxidative stress and lead to the accentuation of hypertension (Latha *et al.,* 2018). Correcting anemia with erythropoietin administration in patients with chronic renal failure might lead to the accentuation of existing hypertension or the development of de novo hypertension (Lakhal-Littleton *et al*., 2015). A cross-sectional study using serum ferritin as an indicator of iron stores showed that serum ferritin levels and the prevalence of hyperferritinemia were increased in men with hypertension compared with normotensive healthy individuals. However, Piperno *et al*., (2012) found that baseline hemoglobin and ferritin concentrations were not associated with changes in BP or incidental hypertension after 5.4 years of follow-up (Galan *et al.,* 2010).

In Nigeria**,** studies have shown that hypertension is a major contributor to 500000 strokes (250000 deaths) and 1000000 myocardial infarctions (500000 deaths) per annum (Matthew *et al*., 2016). Hypertension can lead to damage of blood vessels, these can reduce blood supply to the kidneys and damage the filtering unit in kidneys, result the kidney to stop elimination wastes and extra fluid from the body (Conrad and Umbreit, 2012).

The relationship between body iron store and blood pressure (BP) status has not been well established. Heme iron intake, which is exclusively provided by red meat, poultry, and fish, is positively associated with increased BP. On the other hand, low nonheme iron intake, abundant in fruits, vegetables, and cereal products, is associated with a greater risk of hypertension (Haddad *et al*., 2017). Dietary heme iron represents 2/3 of all absorbed iron, while nonheme iron provides only 1/3, because its absorption is influenced by various foods and nutrients (Jankowich *et al.,* 2016).

There is evidence that coexist that hypertension play a predominant role in the progression of most chronic kidney disease (CKD) and end stage renal disease. However it has been difficult to quantitate the contributions of hypertension to progression of renal disease because of lack of data. Recent studies have shown that inflammation is an important mechanism that determines the death or survival of cells after a severe heart attack (Zhu, 2015). At the same time, the knockout of MHCI in animal studies of cardiac ischemia showed a neuroprotective effect (Adelson, 2012). As a critical component of MHCI, the role of β2M in high blood pressure remains unclear. A study showed that plasma β2M is associated with the occurrence of major adverse cardiovascular events (MACE) in patients with asymptomatic carotid atherosclerosis moreover, plasma β2M is an informative risk marker for both coronary heart disease (CHD) and stroke in postmenopausal women on hormone therapy, and high levels of β2M were associated with an increased risk of ischemic stroke among women (Rist *et al*., 2015). However, there are few clinical studies about the characteristics of β2M in patients with high blood pressure, and a small amount of clinical data discussing the correlation between the levels of serum β2M and high blood pressure.

MATERIALS AND METHODS

SUJECTS

A total of 100 patients with incident(50 males and 50 females) aged 20 and 60 years diagnosed by systolic blood pressure of >/= 140mmHg and Diastolic blood pressure of >/=90mmHg were recruited for the study. 50 apparently healthy non hypertensive were used as the control group. Written Informed consent was obtained from all the subjects.

Blood sample collection Collection: Fresh venous blood (5ml) was collected from the patients by venipuncture using a sterile needle and syringes into clean sterile and plain plastic tubes immediately. The unheamolysed samples in the tubes were centrifuged and separated. The serum samples were stored at -20^{o}c prior to use.

Biochemical Parameters Determination

A. Determination of haemoglobin concentration (Cyanmethaemoglobin method) (Cheesbrough, 2007)

Principle: When whole blood is added to Drabkin's reagent: a solution containing KCN and K3Fe(CN) 6, KCN converts Hb-Fe 2+ (ferrous) to Hb-fe 3+ (ferric) state to form methaemoglobin which then reacts with KCN to form a stable pigment, cyanmethaemoglobin complex. The colour intensity of this mixture is measured in a spectrophotometer at a wavelength of 540nm (or using a yellow-green filter). The optical density (OD) of the solution is proportional to the haemoglobin concentration. All forms of Hb (Hb-C, Hb-O, etc) except Hb-S are measured with this cyanmet-method.

B. Determination of Serum Iron (Baynes, 2016)

An ethanolic solution of thioglycollic acid is used to produce a coloured iron complex, the optical density of which can be measured in any suitable photometer, using either 10 or 20 mm. fused glass cuvettes or matched tubes of 1.1 cm. internal diameter (Baynes, 2016).

C. Determination of Serum Ferritin (Bablok, 2018)

Principle: The method principle for measurement of Ferritin is immuno-turbidimetry using Roche kits on the Hitachi 912 clinical analyzer. Latex bound Ferritin antibodies reacts with the antigen in the sample to form an antigen/antibody complex. Following agglutination, this is measured turbidimetrically. Turbidity formed is proportional to the Ferritin concentration, and is measured at 700nm (primary wavelength) (Bablok, 2018).

D. **Determination of TIBC (Henry 1984).**

Principle:

The unsaturated iron binding capacity is determined by adding Fe (II) iron to serum so that they bind to the unsaturated iron binding sites on transferring. The excess Fe (II) ions are reacted with

ferrozine to form the color complex, which is measured photometrically. The difference between the amount of Fe (II) added and the amount of Fe (II) measured represents the unsaturated iron binding. The total iron binding capacity (TIBC) is determined by adding the serum iron value to the UIBC value.

E. Determination of B2 Microglobulin

Statistical Analysis

Data was analyzed using software statistical package for social sciences (SPSS) version 21. The results were expressed as mean and standard deviation (mean ± SD). Difference in mean values between groups was assessed by student t-test. A test with a probability value of $P< 0.05$ was considered statistically significant**.**

RESULTS

Table 1: Mean Value of Systolic Blood Pressure, Diastolic Blood Pressure and B_2M in male Patients with Incident Hypertension

Parameter	Hypertensive	Normotensive	t-value	p-value
Systole (mmHg)	140.81±5.34	113.30±5.83	3.08	0.006
Diastole (mmHg)	90.82±4.53	75.20±4.05	2.98	0.008
B_2M (ng/ml)	3.38±0.49	2.50±0.27	3.28	0.007

KEY:

B_2M: B_2microglobulin

$P<0.05$; Significant

$p>0.05$: Not significant

Table 2: Mean Value of Systolic Blood Pressure, Diastolic Blood Pressure and B_2M in Female Patients with Incident Hypertension.

Parameter	Hypertensive	Normotensive	t-value	p-value
Systole (mmHg)	138.81±5.34	113.30±5.83	3.08	0.006
Diastole (mmHg)	90.82±4.53	75.20±4.05	2.98	0.008
B_2M (ng/ml)	3.08±0.49	2.50±0.27	3.28	0.007

The mean value of systolic blood pressure was significantly increased (p=0.006) in patients with incident hypertension (120.81±5.34) mmHg when compared to normotensives (113.30±5.83) mmHg.

The mean value of diastolic blood pressure was significantly increased (p=0.008) in patients with incident hypertension (80.82±4.53) mmHg when compared to normotensives (75.20±4.05) mmHg.

The mean value of B_2M was significantly increased (p=0.007) in patients with incident hypertension (3.08±0.49)ng/ml when compared to normotensives (2.50±0.27)ng/ml.

Table 3: Mean Value of Haemoglobin, Iron, Ferritin and Total Iron Binding Capacity in Patients with Incident Hypertension

Parameter	Hypertensive	Normotensive	t-value	p-value
Hb (g/dl)	10.83±0.78	12.941±0.86	5.91	0.000
Iron (µg/dl)	105.28±23.19	122.78±17.94	1.92	0.070
Ferritin (ng/ml)	120.22±24.35	110.37±26.04	0.89	0.381
TIBC (µg/dl)	303.79±14.37	299.68±17.54	0.59	0.562

The mean value of haemoglobin concentration was significantly reduced (p=0.006) in patients with incident hypertension (10.83±0.78)g/dl when compared to normotensives (12.941±0.86)g/dl.

The mean value of iron was significantly reduced (p=0.070) in patients with incident hypertension (105.28±23.19)µg/dl when compared to normotensives (122.78±17.94)µg/dl.

The mean value of ferritin was significantly increased (p=0.381) in patients with incident hypertension (120.22±24.35)ng/ml when compared to normotensives (110.37±26.04)µg/dl.

The mean value of TIBC was significantly increased (p=0.562) in patients with incident hypertension (303.79±14.37)µg/dl when compared to normotensives (299.68±17.54)µg/dl.

Table 4: Correlation of B_2M with Haemoglobin, Iron, Ferritin and TIBC in Patients with Incident Hypertension

Variable	N	R	p-value
Hb	20	-0.41	0.207
Iron	20	-0.02	0.963
Ferritin	20	-0.28	0.399
TIBC	20	0.19	0.574

There was a non-significant negative correlation of B_2M with Hb, iron and ferritin (r=-0.41, p=0.207; r=-0.02, p=0.963 and r=-0.28, p=0.399). There was a non-significant positive correlation of B_2M with TIBC (r=-0.19 p=0.574).

DISCUSSION

Hypertension is a chronic elevation of blood pressure that, in the long-term, causes end-organ damage and results in increased morbidity and mortality (Foe¨x and Sear, 2015).

In the present study, the mean value of B_2M was significantly increased in patients with incident hypertension when compared to normotensives. The increased level of beta2-microglobulin is due to inflammation of which several reports has it that Hypertension is associated with inflammation; however, whether inflammation is a cause or effect of hypertension is not well understood. The result of this study is in agreement with the report by Low *et al*. (2017) who in their study reported a similar finding (Teasdale *et al*., 2017).

Regarding the concentration of haemoglobin in hypertensives it was observed that the mean haemoglobin concentration level in patients with incident hypertension was significantly reduced when compared with normotensives. The result of this study is in contrast with the report from other similar study. A study carried out by Femke *et al*., (2012) reported that haemogbin concentration was significantly raised in hypertensive patient. Hb is strongly related to arterial stiffness, as measured by pulse wave velocity, which, in turn, increases SBP and DBP. Furthermore, free Hb may be a scavenger of nitrogen (II) oxide (NO). NO, produced in the endothelial cells that lines the blood vessels, relaxes the muscle cells surroundings the vessel and thereby controls blood pressure. Another report by Low *et al*., (2017) stated that an obvious mechanism for blood pressure increase with increased Hb levels would be increased blood viscosity. It has been reported that elevation of haematocrit and Hb levels increases blood viscosity and that increased viscosity partly through an effect on blood pressure may worsen cardiovascular function.

In this study, there was no significant difference in the mean value of iron, ferritin and total iron binding capacity in patients with incident hypertension when compared to normotensives. The result of this finding is in agreement with the report by Hafsatu *et al.* (2019), they stated that the serum iron, ferritin and total iron binding capacity levels were higher in patients than in controls but the difference was not statistically significant. The finding on the serum levels of iron, ferritin and total iron binding capacity in this study was also similar to the findings of Conrad and Umbreit, (2011).

The current study revealed that there was no significant relationship between B_2 microglobulin with total iron. The result clearly indicate that B_2 microglobulin level cannot be use to predict the level of iron, ferritin, total iron binding capacity and haemoglobin in patients with incident hypertension. This result of this study is in agreement with the study carried out by Hafsatu, (2019).

Conclusion

Incident hypertension is associated with significant increase in B_2microglobulin level, and haemoglobin concentration which may be predictive of more severe of cardiovascular diseases. There was no significant association between incident hypertension and total iron.

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