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Osmotic Fragility in Essential Hypertension Revisited: A Correlation with Iron Status and Lipid Profile

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Abstract

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Background: Essential hypertension is a major public health associated with increase pressure on the vascular walls and red blood cells (RBCs). In the present work, osmotic fragility (OF) of RBCs was reexamined in the means of its correlation with two risk factor; iron status and lipid profile.

Experimental: OF, iron status parameters, and lipid profile components were measured in 60 patients and compared with the results of 30 controls.

Results: The results showed a significant increase in all iron indices of hypertensive patients in comparing with healthy control group except TIBC, UIBC, and transferrin concentrations, which decrease in these patients in comparing with control group. Serum TGs, total cholesterol, VLDLc, and LDLc were increased while there is no significant in serum HDLc in patients to comparing with control group. There is no significant change in OF between patients and controls where p=0.173. The iron status parameters and lipid profile components were dependent on sex and smoking state. Hemoglobin and PCV were correlated significantly with total cholesterol and LDLc. Transferrin saturation showed a positive correlation with cholesterol, LDLc, and TGs, but negatively correlated with HDL_c. No significant correlation between all the measured parameters and OF in HT patients. There is a significant correlation between serum ferritin and systolic BP and between Hb and systolic BP.

Conclusion: No significant effect on the OF in HT patients. HT patients have elevated level of iron parameters in comparing with controls. OD has no correlation with iron status parameters or with lipid profile components.

Keywords: Iron, TIBC, ferritin, osmotic fragility, hypertension

Introduction

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In overall population of Iraq, the prevalence of hypertension (HT) the prevalence of HT is 4.15% of Iraqi (Iraq National Health Policy 2014). Among the adult population was found to be 40% in 2008. About 50% of total mortality in Iraq is caused by noncommunicable diseases. HT is a major contributor to noncommunicable diseases, a global epidemic which necessitates greater and coordinated efforts by all stakeholders (World Health Day 2013). However, there is a wide variety of the percentage according to the geographic location of peoples. In the north of Iraq, 54.7% out of 1480 participants, were identified as having HT (Saka et al 2019). While in the south of Iraq, a household survey conducted in Thi-Qar Governorate in 2014, revealed that the overall prevalence of hypertension was 26.5% (Al-Ghuzi & Al-Asadi 2014). Therefore, the study of the factors related or affecting the HT is still an importance field of study. One of these factors in the lipid profile. Hypertension and dyslipidemia are the most important risk factors for the occurrence of cardiovascular disease (CVD) (Kotseva et al., 2016). Hypertension and dyslipidemia act synergistically to accelerate CVD progression (Satoh et al., 2015), and more than one half of patients with hypertension have dyslipidemia (Noh et al., 2016). Factors like dyslipidemia that leads to endothelial dysfunction may cause HT. It has a strong association with CVD and abnormal lipid metabolism. Not only the traditional lipid biomarkers, but also the recent lipid components like Apo A1 and Apo B100 (Nayak et al., 2016a).

Another factor that less studied is the effect of mechanical pressure on the Osmotic fragility (OF) of red blood cells (RBCs). OF indicates the proportion or the degree of hemolysis that happens when a sample of RBCs are undergone to osmotic stress such as placed in a hypotonic solution. OF is affected by different factors, including integrity and membrane, cells' sizes, and surface-area-to-volume ratios (Fischbach et al., 2008). The OF test is common in hematology, and is often performed to aid with diagnosis of diseases associated with RBCs membrane abnormalities. OF of RBCs may be considered as a screening test for hypertensive patients who will benefit from diuretic therapy (Fasanmade et al., 1999). OF was increased in other diseases with consequent heart diseases like metabolic syndrome (Kowalczyk et al 2012). Conformation status pf membrane cytoskeleton proteins and membrane lipid fluidity affect erythrocyte shape, size and OF (Kowalczyk et al 2012). The concept that the abnormalities of cell membrane have a role in the pathogenesis of essential hypertension has been proposed by the biochemical studies of erythrocytes and other cells (Aderounmu & Salak 1979; Garay & Meyer 1979; Garay et al 1980).

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However, the subject is neglected during the last decade and very few studies were carried out in this field.

The third parameters to be studied in the HT patients is the iron status. Iron has an important role in maintaining various cellular functions and enzyme reactions; but, iron overload is considered as a risk factor in progression of essential HT (Naito et al., 2011). Iron plays an important role in maintaining physiological homeostasis in the body; however, elevated iron can lead to free radical generation, resulting in tissue damage (Naito et al., 2011). Iron is stored primarily in the ferritin protein. Ferritin, is a protein regulating iron homeostasis, which is a widely used to evaluate iron status and is especially important for detecting iron deficiency (Sun et al., 2008). Serum ferritin, but not iron level, was considered as a significant predictor of hypertension in middle-aged Korean men (Kim et al., 2012). Hydrogen peroxide and organic hydroperoxides react with hemoglobin to release iron which promotes Fenton reaction and DNA degradation (Gutteridge 1986). In the Fenton reaction, Fe(II) catalyzes the formation of reactive hydroxyl radicals. Interaction with lipids may initiate the formation of oxidized LDL that ultimately leads to the development of foam cells and progression of CVD including HT. It was published that high iron stores (hyperferritinemia) is associated with aortic stiffness and cardiac diastolic impairment (Valenti et al., 2015). Oxidative stress has been considered as a pathogenic factor associated with essential hypertension (Rodrigo et al., 2013). Oxidative stress has a vital role in the development of hypertension (Rodrigo et al., 2011). Redox imbalance leads to the activation of a number of signaling pathways arranged by reactive oxygen species (ROSs) and reactive nitrogen species (RNSs) (Elahi et al., 2009).

In the present study, an attempt is carried out to define the effect of HT on the OF, lipid profile, and iron status in Iraqi hypertensive patients. The aim is to obtain a recommendation about the risk of changes in the OF according to the indices as a marker for subsequent ischaemic heart diseases.

Subjects and Methods

This study was performed at Al-Kufa University, College of science in period between May 2018-June 2019. This study was designed to examine the association between fragility, iron status, and lipid profile on 88 patients with uncomplicated essential hypertension and have no other systemic diseases with mean age 49±13 years old. HT was diagnosed according to the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC) guidelines (Mancia et al

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2013)-systolic blood pressure ≥ 140 mmHg and/or diastolic ≥ 90 mmHg. Each hypertensive subject had a blood pressure measurement by conventional sphygmomanometry in excess of 95/140 mmHg (seated posture), with the arm in the horizontal position after five minutes of quiet sitting, as well as 30 healthy males as a control group was also enrolled in this study for comparing purposes with normal blood pressure and their aged range was between 46±14 years old. The control group was confirmed to be normal by biochemical and hematological examinations. Exclusion criteria included a history of infection, inflammation, cancer, diabetes mellitus, and congestive heart failure. Biochemical, hematological, all clinic-pathological data of the patients were collected from the clinical files of the patients. Body mass index (BMI) was calculated as weight (kg) divided by the square of height (m^2).Informed consents were taken from all the participants before participation in the current study. All procedures were in accordance with the established ethical standards. The Ethics Committee of the University of Kufa approved the study protocol (512/2018).

Measurements: Blood samples were collected from individuals in the morning after r at least 12 hours overnight fasting and put in a plain tubes for serum separation by centrifugation in order to estimate the iron status parameters and hormones level. Other part of the fresh blood were put in the EDTA tube for Serum total cholesterol and TGs were measured by enzymatic colorimetric methods using the kits supplied by Biolabo®, France. HDLc level was measured after precipitation of all other lipoproteins phosphotungstic acid precipitant kit supplied by Biolabo®, France.

Very low density lipoprotein (VLDLc) were estimated using the following formula:

VLDLc = TG (mmol/l)/2.19

Low density lipoprotein (LDLc) were estimated using the following formula:

LDLc= TC(mmol/l)- VLDLc (mmol/l)- HDLc(mmol/l)

Different Atherogenic indices were calculated by dividing cholesterol, TGs, or LDLc by HDLc.

Serum levels of iron were estimated using Ferrozine® colorimetric method, total Iron Binding Capacity (TIBC) were estimated colorimetrically by the following procedure: An excess of iron is added to the serum iron to saturate the transferrin. The unbound iron is precipitated with basic magnesium carbonate. After centrifugation, the iron in the supernatant was determined. Unsaturated iron-binding capacity (UIBC), the amount of protein (apotransferrin) still available to bind iron, can be estimated from the formula: UIBC=TIBC - Serum iron. The ferritin quantitative test is based on a solid phase enzyme-linked immunosorbent assay (ELISA). The assay system

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utilizes one rabbit anti-ferritin antibody for solid phase (microtitre wells) immobilization and a mouse monoclonal anti-ferritin antibody in the antibody-enzyme horseradish peroxidase (HRP) conjugate solution.

Transferrrin saturation percentage (TS%) was calculated from the following equation (McLaren et al 2001):

$$TS\% = (\text{Serum Iron}/TIBC) * 100\%$$

Transferrin concentration can be calculated using the following formula (Kennedy et al 2004):

$$\text{Transferrin Conc. (g/L)} = \text{S.Iron } (\mu mol/L) / (TS\% * 3.98)$$

The formula is based on the maximal binding of 2 mol Fe^{3+}/mol of transferrin and a molecular weight of 79,570gm/mol for transferrin (Kennedy et al 2004).

Biostatistical Analysis

The student T-test was employed to assess differences in scale variables between diagnostic categories and analysis of contingency tables ($\chi 2$-test) was used to check associations between nominal variables. Associations among variables were computed using Pearson's product-moment and Spearman's rank-order correlation coefficients. All tests were 2-tailed and a p-value of 0.05 was used for statistical significance. All statistical analyses were performed using IBM SPSS windows version 25, 2017.

Results

Comparison in the characteristics of study groups

Table 1 indicated that the patients groups were more smokers and employment and less educated than the control group. Blood pressure, as expected, is higher in patients than controls. No significant difference between groups in age and sex.

Comparison in iron status parameters between HT patients and controls

The results of iron indices expressed as mean ± standard deviation are presented in Table 2. There is a significant increase ($p<0.05$) in all iron indices of hypertensive patients in comparing with healthy control group except TIBC, UIBC, and transferrin concentrations, which decrease in these patients in comparing with control group.

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Comparison in Lipid Profile between HT patients and controls

The results in Table 2 showed a significant increase in serum TGs, TC, VLDLc, and LDLc in patients comparing with control group. While there is no significant in serum HDLc in patients to comparing with control group.

Comparison in OF between HT patients and controls

There is no significant change in fragility between patients and controls where (p value =0.173) as presented in Figure 1.

Comparison between male and female

Table 3 showed the comparison between iron indices in male and female patients. There is a significant increase ($p<0.05$) in all iron status parameters in male group as compared with female group except TIBC, UIBC, and Tf which showed a decrease in male group. TG, VLDLc, and TG/HDLc were lower in female group in comparing with male group. While all other lipid profile parameters were differ insignificantly. There is no significant change in fragility between male and female patients.

Effect of smoking on the parameters

The results of all the measured parameters in smokers and non-smokers patients in addition to p-values of the comparison between both groups represented in Table 4. All iron status biomarkers were increased ($p<0.05$) in smoker HT patients in comparison with non-smoker patients except TS% and UIBC which reduced in smoker patients. TIBC showed no significant difference between groups. No significant difference in lipid profile parameters between smokers and non-smoker HT patients. There is no significant change in fragility between smokers and non-smokers patients.

Correlation between parameters

To obtain an indication about the usefulness of iron indices as risk factor for CHD, correlation coefficients (r) between iron indices and lipid profile components were calculated. No correlation was found between serum iron and the variables of lipid profile. Hb and PCV were correlated

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significantly with total cholesterol (r=0.288, p=0.018) and LDLc (r=0.271, p=0.021). UIBC were correlated significantly with total cholesterol (r=0.274, p=0.016) and LDLc (r=0.277, p=0.021). Transferrin saturation was found to maintain strongly positive correlation with total cholesterol (r=0.323, p=0.004), LDLc (r=0.310, p=0.008), and TGs (r=0.283, p=0.018), but strongly negative correlation with HDL_c (r=-0.282, p=0.022). No significant correlation between all the measured parameters and OF in HT patients. There is a significant correlation between serum ferritin and systolic BP (r=0.317, p=0.014) and between Hb and systolic BP (r=0.247, p=0.037).

Discussion

The major finding in Table 1 is the higher smokers among HT patients in comparing with control group. Previously, smoking was found as one of the major risk factors for hypertension in addition to alcohol consumption, obesity, dyslipidemia, and dietary pattern (Bhavani et al., 2003). The multivariate analysis identified age, male, nonemployment, and obesity as the statistically significant factors associated with hypertension (Saka et al 2019). Accordingly, Table 2 results indicated increase in most iron status parameters in HT patients which is in accordance with Fatemeh et al., (2013) study that found the increased levels of serum iron and ferritin might consider as risk factors for development of HT in combination with other risk factors (Fatemeh et al., 2013; Al-Hadrawy et al 2014). Plasma ferritin, transferrin saturation, total iron-binding capacity, or serum iron were used as objective markers for body iron stores. Of these biomarkers or indices, ferritin is considered the best single indicator of total body iron (Corti et al 1997). The significant elevations in the all parameters of iron indices inpatients group as compared with the control group except TIBC, UIBC, and transferrin concentrations, which decrease in these patients in comparing with control group are in accordance with many other researches (Kim et al 2012; Lee et al 2018). Recently, Zhu et al (2019) showed that the incidence of HT is related to the increase in iron status parameters especially Hb and Tf level. Their results suggests that dysregulation of iron metabolism is an important independent risk factor for the onset of HT (Zhu et al 2019). RBCs are highly susceptible to oxidative damage due to the high concentration of oxygen and hemoglobin, it is considered as a powerful promoter of the oxidative process (Pandey & Rizvi 2010). Otherwise, Oxidative stress and inflammation are cooperative events involved in the pathogenesis of HT. Iron accumulation in macrophages within the arterial wall has been hypothesized to induce oxidative stress and the release of proinflammatory mediators (Sullivan et

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al., 2007). Iron catalyzes the formation of reactive oxygen species through the Fenton and Haber-Weiss reactions (Verschuren 1995). Free radicals cause lipid peroxidation, leading to the modification of LDL at the molecular level, facilitating its deposition and leading to the formation of atherosclerotic plaque (Navab et al 1995). Generation of reactive oxygen species (Horwitz et al 1994) has been proposed as a mechanism for reperfusion injury.

The results of lipid profile in Table 2 indicated a dyslipidemia state in HT patients in comparing with controls. Dyslipidemia plays a role in endothelial dysfunction which is central in the pathogenesis of atherosclerosis, insulin resistance, thrombosis, as well hypertension (Nayak et al., 2016). This study exhibited that there were a significant elevations in the serum levels of TG, cholesterol, VLDLc, LDLc in patients group with HT as compared with the control group, and these findings were concordant with the previous studies (Nayak et al., 2016; Kudhur & Shatha, 2018). Many researches have also found a positive correlation between blood cholesterol levels and CVD; thus, a decrease in cholesterol levels can significantly reduce the CVD risk (Al-Dejal et al 2014; Piepoli et al., 2016).

Atherogenic ratios of HT patients and control groups were used to estimate the risk of CVDs. Some studies demonstrated that the TC/HDLc and the LDLc/HDLc ratios are better predictors of atherosclerosis and CVD than any other single lipid marker (Zhan et al 2014). Likewise, the TG/HDLc ratio was demonstrated to be as significant a predictor of CVD as the two other lipid ratios (Zhan et al 2014). Hence, the study of lipid profile in hypertensive is of importance matter to determine the risk of CVDs in those patients. It is hypothesized that it is the ratio of TG/HDLc in the plasma that determines the esterification rate of cholesterol (Upadhyay 2015). Furthermore, increased TG/HDLc ratios also indicate the presence of atherogenic small, dense LDL particles (Maruyama, et al 2003), could serve as a good predictor of myocardial infarction and the presence of coronary atherosclerotic lesions (Burns et al 2012).

There is no significant difference in OF between patients and control groups as seen in Figure 1. The slight difference in the results of OF, even it is insignificant ($p > 0.05$), may be due to the statistical variability that depends on the sample size, technical, methods differences and individual differences within the sample such as age, sex, type of treatment and duration of disease. Other studies are found the normal cell swells when placed in a 0.6% saline solution. Normally hemolysis begins at 0.42%, when the red blood cell (RBC) volume increases to ~145% of normal. In a 0.35%

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saline solution, the cells are fully hemolyzed. RBC volume at this concentration, before it bursts, is about 165% of normal (critical hemolytic volume) (Kumar 2002). OF in patients with essential hypertension more increased than that of the normotensive controls. These results show the increased lability of erythrocytes membranes in essential hypertension (Tsuda et al 1984). RBCs deformability was found decreased in essential hypertension patients versus the normal. A direct correlation was revealed between red cell mechanical resistance (from the time of hemolysis) and the value of arterial pressure in essential hypertension (Dudaev et al 1990). In one study, a high variation in the OF of the erythrocytes by Ca-loading in patients with essential HT which might be due to the abnormality of Ca-handling of the cell membranes leading to an increase in the intracellular Ca concentration, contributing to the pathogenesis of essential HT (Tsuda et al., 1986). The slight difference in the results of OF, even it was insignificant ($p > 0.05$). The explanation of this unexpected finding might be due to the smaller sample size enrolled in this study, technical method differences and individual differences within the sample such as age, sex, type of treatment, and duration of disease. Another explanation for this non-significant difference between patients and control groups might be the control group has family history of hypertension. High OF was present in patients with essential hypertension and normotensive subjects with family history of hypertension, as compared with normotensive controls without family history of hypertension (Tsuda et al., 1984). Thus, the OF might reflect structural and functional abnormalities of cell membranes, and could be one of the genetic biomarkers of the hypertensive predisposition. Furthermore, abnormalities of the physical properties of the membrane and of multiple transport systems have been implicated in the pathogenesis of hypertension (Russo et al 1997). There is evidence that the sodium hydrogen exchanger is stimulated in hypertensive patients either by an increased cellular calcium load or enhanced external calcium entry. An increased Na^+/H^+ exchanger could play a significant role in the pathogenesis of hypertension, both by stimulating vascular tone and cell growth and possibly by increasing sodium reabsorption in renal proximal tubule cells (Soleimani et al 1995).

In Table 3, it is expected that the iron status is more consistent in male in comparison with female group due to the menstruation state and usual lower Hb in female in comparing with males. As women become older, the CVD incidence increases gradually, largely narrowing the gender gap, and that there is a parallel increase in body iron store (Sullivan 1993). In the present work, there is no significant difference between male and female HT patients in OF values. In one study,

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it is found that the OF seemed to be slightly increased in females compared with that in males (Tsuda et al 1984). Our result indicated no correlation between gender and OF in HT patients.

All iron status biomarkers were increased ($p<0.05$) in smoker HT patients in comparison with non-smoker patients except TS% and UIBC which reduced in smoker patients as seen in Table 4. Hydroxyl radical generated by aqueous cigarette tar can cause oxidative damage. Cigarette smoke, respirable fibres and dusts act synergistically in the increasing production of damaging hydroxyl radicals. Cell death induced by cigarette smoke exposure can largely be accounted for an enhancement in oxidative stress (Jiayuan et al., 2012). It is well documented fact that cigarette smoke is a potent source of oxidative stress. Smoking is a risk factor for coronary artery, peripheral vascular and cerebrovascular diseases. Smoking causes endothelial dysfunction, atherosclerosis and arrhythmias through the combined effects of nicotine, carbon monoxide and polycyclic aromatic hydrocarbons (Salahuddin et al 2012). Serum iron and red cell hemoglobin concentration were increase in chronic cigarette smokers patients when compared with nonsmoking patients. These results are in agreement with previous studies (Malenica et al 2017). As tissue hypoxia leads to inadequate oxygenation of blood circulation through lungs results in erthrocytosis and consequent increased production of erythropoietin (El-Zayadi, et al 2002).

Increased total red blood cell mass count increases the number of destroyed RBCs in the normal turnover process which subsequently increases iron over load (Malenica et al 2017). Significantly increase in serum ferritin with smoking patients may be due to increase iron in smokers patients. Other studies were found the smoking has been shown to be associated with an increased level of serum ferritin (Lee et al 2016).

Serum indices of iron homeostasis revealed disparities between nonsmokers and smokers. Relative to nonsmokers, serum iron and ferritin concentrations and transferrin saturation in cigarette smokers were significantly increased (Ghio et al 2008.). There is a significant decrease in serum TIBC and serum UIBC in smoking and nonsmoking patients ($p<0.05$). These result according with Saudi population which serum TIBC and serum UIBC were statistically decreased in smoking comparing with nonsmoking (Al-Malki et al 2009).

The mean lipid profile parameters indicated an increase in smokers group in comparing with nonsmoker group even this difference is statistically insignificant ($p>0.05$) as seen in Table 3. This results may be due in part to the parallel significant increase in both groups in comparing

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with control group and the increase in these cases may follow up the same factors affecting lipid profile in hypertension. The other cause may be due to the small number of smokers in comparison with nonsmoker individuals. In one research, it is found that cigarettes lead to increase in the concentration of serum total cholesterol, TGs, LDLc, VLDLc and fall in the levels of antiatherogenic HDLc (Śliwińska-Mossoń et al 2014). There is no significant change in fragility between smokers and non-smokers patients indicating lack of direct effect of nicotine on the OF in HT and control groups.

The correlation study indicated that Hb, PCV, and UIBC were correlated significantly with total cholesterol and LDLc. Transferrin saturation was found to maintain strongly positive correlation with total cholesterol, LDLc, and TGs, but strongly negative correlation with HDL_c. No significant correlation between all the measured parameters and OF in HT patients. These results may be due to the role of free iron on the lipid peroxidation and other related biological processes. The general mechanism that Fe(II) and certain its chelates react with lipid hydroperoxides (ROOH) and splitting the O-O bond to produce RO• which can also abstract H• from polyunsaturated fatty acids and from ROO• radicals that can continue propagation of lipid peroxidation (Minotti & Aust 987).

It is suggested that the catalytic role of iron in lipid peroxidation may be an important factor in the formation of atherosclerotic lesions. Normal native LDLc can cross the arterial wall without causing damage to the vessel wall. Iron-catalyzed free radical reactions cause oxidation of LDLc, which occurs in endothelial cells, smooth muscle cells, lymphocytes, or macrophages (Halliwell & Chirico 1993).

In our study, there was a significant correlation between the serum total lipids and Fe. Some researchers found that iron deficiency is linked to low serum lipid level and Fe has been shown as a potential risk factor for hyperlipidemia in humans (Burtis et al 1994). However, Sulibursk a et al (2011) found no significant correlation between the serum or diet concentration of iron and the serum cholesterol or TGs level (Sulibursk a et al 2011). It is found previously that Hb concentrations were positively associated with the risk of incident hypertension (Shimizu et al 2014). Other studies reported that Hb showed a significant positive association with blood pressure (Deinum 2012; Rasmussen et al 2016).

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Iron overload was shown to augment the formation of atherosclerotic lesions in hypercholesterolemic rabbits, (Araujo et al 1995) probably by stimulating LDLc oxidation in the arterial intima or by influencing lipoprotein synthesis in the liver, which could lead to increased susceptibility to oxidation in the intima (Linpisarn et al 1991). Due to increase the disorders in the structure of erythrocyte cytoskeleton proteins, the increase in membrane lipids fluidity may causes the increased erythrocyte OF (Kowalczyk et al 2012). RBC membranes from hypertensives have an increased cholesterol: phospholipid ratio in association with high sodium lithium transport (Villar et al 1996) and increased ratios of fatty acid metabolites to precursors compared to those from age matched normotensives (Russo et al 1997). Such changes in lipids produce a high membrane microviscosity and decrease in fluidity which may be responsible for increased permeability to sodium and other alterations in sodium transport (Carr et al 1995). The high polyunsaturated fatty acid content of the red blood cell membrane and the continuous exposure to high concentration of oxygen and iron in hemoglobin are factor which make RBCs very sensitive to oxidative injury (Kusmic et al 2000). The biomechanics of erythrocytes, determined by the membrane integrity and cytoskeletal structure, provides critical information on diseases. Such release profile reflects sensitively the changes in erythrocyte fragility induced by chemical, heating, and glucose treatment (Zhan et al 2012). The RBCs membrane fragility indicate a decrease in the membrane flexibility (increased fragility) of hyperlipidemic, which may be due to the disturbance of ionic motion through the membrane and /or the change in the molecular properties of the macromolecules forming the membrane. This change in the ionic mobility through the cellular membrane leads to changes in metabolic function and may causes changes in OF. OF was higher in hypertensive subjects than in normotensive subjects. The OF also became similar in the two groups with control of blood pressure in the hypertensive group. OF may be determined as a screening tool for hypertensive patients who will benefit from diuretic therapy (Fasanmade 1999). The RBCs size was larger in hypertensive subjects than in normal controls. (Fasanmade 1999). The cause may be due to the fact that mean erythrocytic zeta potential of control group was higher than hypertensive patients (Gaikwad & Avari 2017). The data suggest that there are morphological changes in erythrocyte structure, increased OF value as compared to that of healthy volunteers which may be the major cause for progression to the development of CVD (Gaikwad & Avari 2017).

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Conclusion

Blood pressure has no significant effect on the OF in HT patients. HT patients have elevated level of iron parameters in comparing with controls. OD has no correlation with iron status parameters or with lipid profile components. Increased iron level causes oxidation and damage to artery wall leading to increased risk of placing cholesterol in walls and subsequently increase risk of coronary artery disease. OF has no role in HT while dyslipidemia, iron status are considered as important complementary parameters for evaluation of HT. Further studies required for other biochemical parameters in larger patients sample size.

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Table 1: Characteristics of HT patients and control group.

Iron indices	Patients	Control	p-Value
Age Year	49.13±9.33	46.49±6.88	N.S.
Sex (M/F)	40/48	15/15	N.S.
Education (Y/N)	63/25	3/27	0.003
Employment (Y/N)	71/17	12/18	0.026
Diastolic BP mmHg	97.27±11.63	79.88 ± 3.14	<0.001
Systolic BP mmHg	158.38±14.74	120.63±4.14	<0.001
Smoking (Y/N)	34/56	4/26	<0.001
BMI kg/m^2	28.27±2.32	27.63±2.14	N.S.

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Table 2: Iron indices and lipid profile components in patients and control groups

Iron indices	Patients	Control	p-Value
Hb g/dL	13.44±2.11	12.65 ±1.13	N.S.
PCV %	43.31± 6.32	40.94 ±3.39	N.S.
Ferritin pM	(383.17)	(166.08)	<0.001
S.Iron µM	21.67± 6.21	17.82 ±4.37	0.026
TIBC µM	51.91±10.96	53.17 ± 12.83	N.S.
TS %	(42.87)	(32.48)	<0.01
Transferrin mg/L	(127.00)	(84.21)	0.008
UIBC µM	30.07± 6.18	35.49 ±9.11	<0.001
TG mM	2.50 ±0.90	1.69 ±0.43	<0.001
Cholesterol mM	5.59 ±1.24	4.44 ±0.64	<0.001
HDLc mM	1.04 ±0.44	1.06 ±0.24	N.S.
VLDLc mM	1.14 ±0.41	0.77 ±0.19	<0.001
LDLc mM	4.35 ±1.35	2.76 ±0.83	<0.001
TC/HDLc	6.89±3.32	4.37 ±1.36	<0.001
TG/HDLc	2.81±1.65	1.65 ±0.47	<0.001
LDLc/HDLc	4.55 ±2.72	2.73 ±1.30	0.231

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Table 3: Serum level of the measured parameters in male patients in comparison with female patients. Median in brackets.

Iron indices	Male Group	Female Group	p-value
Hb g/dL	14.38 ±2.45	12.78 ± 1.86	0.036
PCV %	45.21 ± 5.88	38.12 ± 4.19	0.012
Ferritin pM	(411.25)	(178.71)	<0.001
S.Iron µM	18.81± 4.27	14.38 ± 5.09	0.028
TIBC µM	39.67± 7.17	56.19 ± 10.76	0.037
TS %	(46.14)	(25.59)	0.008
Transferrin mg/L	102.43	141.19	<0.001
UIBC µM	21.69 ± 3.47	42.44 ± 5.71	0.017
TG mM	2.62 ± 0.64	2.01 ± 0.77	0.014
Chol mM	6.11 ± 1.43	5.97 ±1.38	0.076
HDLc mM	1.08 ± 0.37	1.03 ± 0.51	0.317
VLDLc mM	1.20 ± 0.29	0.92 ± 0.35	0.014
LDLc mM	3.83 ± 0.77	4.02 ± 0.52	0.082
TC/HDLc	5.66 ± 3.87	5.80 ± 2.71	0.094
TG/HDLc	2.43 ± 1.73	1.95 ±1.51	0.111
LDLc/HDLc	3.55 ± 2.08	3.90 ± 1.02	0.214

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Table 4: Serum level of the estimated parameters in smoker and non-smoker hypertensive patients.

Iron indices	Smokers	Non-Smokers	p-Value
Hb g/dL	14.27 ± 2.48	13.35 ± 1.9	0.034
PCV%	44.31 ±8.48	43.05 ± 5.68	0.031
Ferritin pM	342.14 ± 338.25	251.66 ±190.34	0.008
S.Iron uM	21.99 ±13.92	18.87 ± 5.78	0.002
TIBC µM	51.91±10.96	53.17 ± 12.83	0.471
TS%	55.94 ± 37.14	47.81 ±15.90	0.008
Transferrin g/L	108	113	0.906
UIBC uM	28.92± 30.08	21.79 ± 13.26	0.001
TG mM	2.51 ± 0.66	2.50 ± 0.95	0.075
Chol mM	6.36 ± 1.30	5.92 ±1.34	0.755
HDLc mM	1.09 ± 0.43	1.02 ± 0.44	0.601
VLDLc mM	1.15 ± 0.29	1.14 ± 0.44	0.075
LDLc mM	5.14 ± 1.50	4.79 ± 1.33	0.742
TC/HDLc	7.15 ± 4.19	6.86 ± 3.11	0.117
TG/HDLc	2.81 ± 1.87	2.82 ±1.62	0.818
LDLc/HDLc	4.74 ± 3.50	5.65 ± 3.01	0.755

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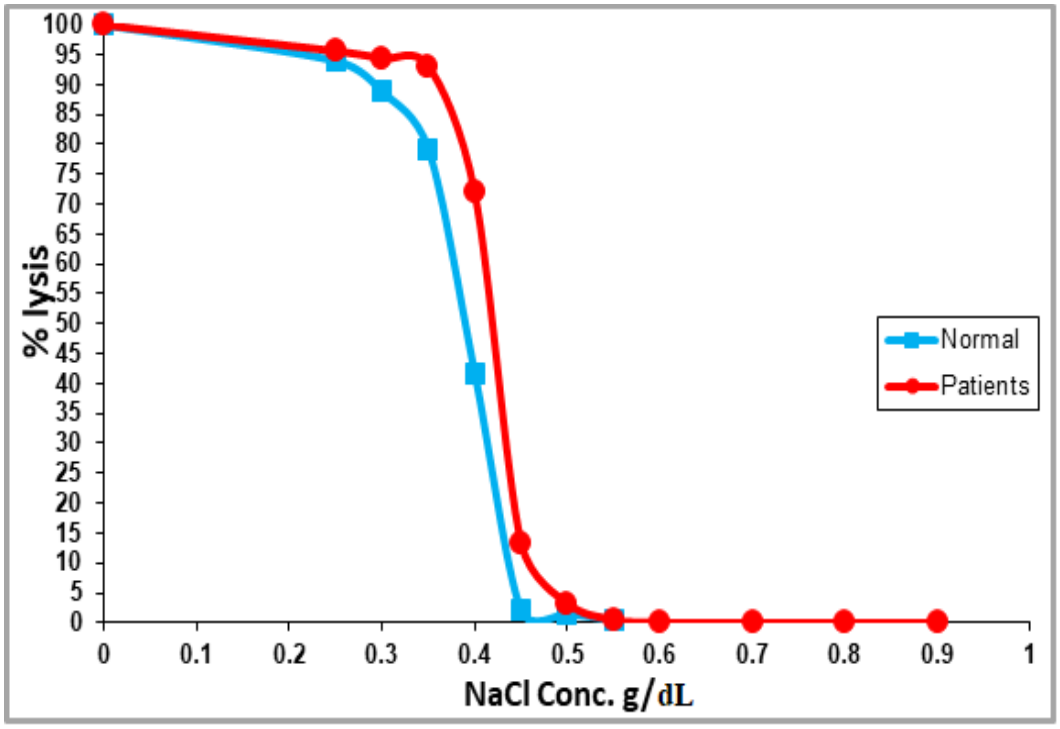

Figure 1: Normal osmotic fragility curve with left shift in hypertensive patients group. p-value of the comparison=0.173.