ORIGINAL ARTICLE

LEVEL OF FETUIN-A, ENDOTHELIN-1 AND SEX HORMONES IN SERUM OF ISOLATED SYSTOLIC HYPERTENSIVE PATIENTS

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Background: Arterial stiffness is characterised by isolated systolic hypertension (ISH). Factors such as Fetuin-A, Endothelin-1 (ET-1), Testosterone (TEST), and Progesterone (PROG) have been demonstrated to be involved in arterial stiffness. If changes in blood level of these parameters are detected in ISH and hypertensive patients, it can reflect their expression, production, and association with the pathophysiology of these diseases. Since this association of ISH and hypertension (HTN) with above mentioned factors is still not clear, the present study was carried out for their identification in such diseases. Methods: Serum level of these parameters was measured through ELISA in three groups: (I) ISH patients (n=9), (II) hypertensive patients (n=7), and (III) normal subjects (n=7). Results: Significantly reduced levels of Fetuin-A (0.17±0.03 ng/ml, n=39) were observed in ISH (p<0.05) without significant changes in TEST (19.2±1.9, n=15), PROG (0.72±0.2, n=19), and ET-1 (0.09±0.02, n=30) levels compared to normal subjects. However, in HTN, increased ET-1 (0.2±0.03, n=14, p<0.05) and decreased TEST (8.125±1.8, n=9, p<0.05) with non-significant changes in Fetuin-A (0.29±0.06, n=18) and PROG (0.67±0.3, n=7) levels have been observed. Conclusion: Low levels of Fetuin-A, in ISH suggests its possible contribution in the pathophysiology of arterial stiffness and subsequently causing increase in systolic blood pressure. An increase in the (ET-1) endothelin-1, and decrease in TEST in hypertensive patients suggests their possible involvement in physiology and pathophysiolo of these vascular diseases. These findings open new avenues for further studies regarding pathophysiology of ISH and HTN that may lead to finding a better treatment for these diseases. Keywords: Isolated systolic hypertension, arterial stiffness, Fetuin-A, endothelin-1, testosterone

INTRODUCTION

Demographically, more than a quarter of the total Saudi Arabian population has been reported to be affected with hypertension (HTN). The patients, who are elder, especially above 50 years, are in favourable age to develop ISH. Studies revealed that an elevated systolic blood pressure (SBP) is an important predictor for cardiovascular diseases such as coronary artery disease, congestive heart failure, and stroke, than the elevated diastolic blood pressure (DBP).

Arterial stiffness is one of the most important factors for the characterisation of ISH. The exact mechanisms which are responsible for producing arterial stiffness followed by development of ISH are yet to be understood properly. However in previous studies, several factors have been demonstrated to be associated with arterial stiffness such as liver-produced anti-calcificatory hormone, Fetuin-A, endothelial-derived vasoconstrictor, ET-1, and the sex hormones, Testosterone (TEST) and Progesterone (PROG). Changes in the blood level of these parameters in hypertensive patients, when both SBP and DBP are higher than normal, might reflect the level of their expression and production, for the initiation of the pathophysiologial events that can lead to development of ISH and/or HTN. However, deeper studies are needed to confirm it. Additionally, in the current era of development and identification of drugs for the treatment of various cardiovascular diseases, the identification and characterisation of drugs that can effectively reduce the elevated SBP is yet difficult. Obviously, the selection of effective drug especially for ISH will only be successful, if the actual factors involved in its pathophysiology are fully understood.

The objective of this study was to examine the hypothesis that the actual cause of ISH that occur as a result of arterial stiffness may be the abnormal expression and production of vasoactive factors such as Fetuin-A, ET-1, TEST and PROG.

MATERIAL AND METHODS

Patients (aged 40–80 years) with ISH, hypertension and normal subjects were selected and data regarding their age, gender, lifestyle such as smoking, physical activity, salt intake habits, and medications were recorded. Patients with diabetes, chronic inflammatory arthritis, sickle-cell anaemia and β-thalassaemia were excluded. Written informed consent of the participants and approval of local research and ethics committee were obtained.

Blood pressure was recorded thrice to get an average. Blood samples were collected from the patients as well as normal subjects in Alnoor Specialist Hospital,
Makkah, Saudi Arabia. These samples were evacuated in serum separating tubes (SST), which were kept in ice, light protected and transported immediately to the physiology laboratory in Umm Alqura University to be centrifuged at 1000 rpm for 15 min. Serum was separated and immediately stored in eppendorf tubes (at -86 °C) until tested.

ELISA kits obtained from USCNLIFE, China were used for Fetuin-A, endothelin-1, testosterone and progesterone. The measurement of Progesterone and Testosterone were performed in King Abdulaziz University Hospital by Modular Analytics E170.

The serum level of the measured parameters was expressed as Mean±SEM. Comparisons between groups was made using Student’s t-test, and p<0.05 was taken as significant.

RESULTS

Serum level of TEST was 45% lower in patients with HTN (8.125±1.8, n=9) compared to normal subjects (15±2.9, n=10, p<0.05). Similarly the level of TEST was also 58% lower in hypertensive patients (n=9) compared to patients with ISH (19.2±1.9, n=15, p<0.05). However, there were non-significant differences in the level of TEST between ISH patients and normal subjects (p>0.05) as shown in Figure-1.

Serum level of PROG was statistically similar in ISH patients (0.72±0.2, n=19) compared to normal subjects (0.5±0.1, n=7, p>0.05). There were no statistical differences between the level of PROG in ISH patients compared to hypertensive patients (0.67±0.3, n=7, p>0.05). Comparison between the level of PROG in hypertensive and normal subject also showed non-significant differences (p>0.05) as depicted in Figure-2.

Serum level of calcification inhibitor hormone, Fetuin-A, was 54% lower in patients with ISH (0.17±0.03, n=39) compared to normal subjects (0.37±0.07, n=20, p<0.05), and there were no statistically significant differences between hypertensive patients (0.29±0.06, n=18) and normal subjects (p>0.05). However, comparison between hypertensive and ISH patients demonstrated significantly different levels of Fetuin-A, being 41% lower in ISH than HTN (p<0.05) as shown in Figure-3.

Serum level of ET-1 was 100% higher (0.2±0.03) in patients with HTN (n=14) compared to normal subjects (0.1±0.03, n=14, p<0.05). ET-1 was also 1.2-fold higher in hypertensive patients compared to patients with ISH (0.09±0.02, n=30, p<0.05). However, comparison of ET-1 levels between ISH patients and normal subjects demonstrated non-significant (p>0.05) differences as shown in Figure-4.

DISCUSSION

The present study demonstrates significant reduction of TEST level in HTN which is not changed significantly in ISH compared to normal subjects. It suggests that TEST may or may not be involved in the pathophysiology of arterial stiffness and doubts are there that it could affect the systolic blood pressure or not. Phillips et al11 also reported decreased TEST levels (fasting and free) in men suffering from HTN. However, the physiological importance of this finding is still controversial since, androgens have diverse, even contradictory effects on the cardiovascular and renal vascular system.10 A number of reports13-14 favour these
findings of reduced TEST levels in HTN. In one of these reports, acute administration of TEST has been described for its vasodilator properties that also lowers peripheral vascular resistance, reduces cardiac afterload, and increases cardiac index. Additionally, my finding of reduced TEST levels in HTN is also supported by the study on thickening effects of low TEST levels on vascular wall (intima media), reported for the common carotid artery. Men with proven coronary atherosclerosis have also been reported for lower levels of TEST. It is also to be noted that TEST deficiency has also been associated with various pathophysiological conditions in men that alters endothelium function, vascular smooth muscle reactivity, and lipid profiles leading to mortality in men.

Even in the presence of some contradictory findings as quoted above in relation with the vascular walls of kidney, it is clear that TEST principally demonstrates its role in regulation of blood pressure and atherosclerosis process. Therefore, the down-regulation of TEST in HTN, but no change in ISH observed in this study probably indicate either detrimental or compensatory physiological mechanism that needs further studies in depth to elucidate the exact role of TEST on vascular structure and its role in various types of hypertension.

The level of PROG did not change in ISH and HTN patients compared to normal subjects, suggesting that the pathophysiology of arterial stiffness and subsequently increased systolic blood pressure may not involve change in the level of this female sex hormone and thus, pathophysiology of ISH as well as HTN are probably not related with change in PROG level. However, Natoli et al. have reported promotion of elastic degradation when human aortic smooth muscle cells were cultured with PROG. They suggested for an eventual contribution of PROG in an increase in arterial stiffness. In my opinion, if PROG can increase arterial stiffness in smooth muscle wall, then it might also be possible that it can decrease the vascular compliance leading to HTN. But, my results do not support this suggestion of Natoli et al. on the basis that in my study, no significant change was observed in serum PROG levels in HTN compared to normal subject.

My results regarding decreased TEST levels and unchanged PROG levels in HTN, probably reflect difference in the role of both the hormones in relation to HTN although, both of these hormones are steroid in their chemical nature. It is important to note that deficiency of another steroidal (female reproductive) hormone, oestrogen (EST) has been reported for impaired regional blood flow, low systolic blood pressure and heart rate. In the light of this study, it can also be suggested that two of the major female and male reproductive hormones, i.e., PROG and TEST, probably having some structural implications (being steroidal hormones), which affect differently on the vascular walls in different physiological or pathophysiological conditions. Therefore, the difference in the pathophysiological condition and mechanism that exists between HTN and ISH might be one of the reasons that both the TEST and PROG showed difference in their levels in my study when HTN and ISH are compared with normal subjects. In addition, on the basis of the role of various male and female reproductive hormones reported in relation with HTN, ISH (including this study) and other vascular ailments, especially in kidney it is true that now TEST, PROG and EST are not just a sex hormone anymore.

The present study demonstrates that serum level of Fetuin-A is reduced in patients with ISH as compared to hypertensive and normal subjects. It is suggested that since Fetuin-A is an important anti-calciﬁcation hormone; its reduction in ISH patients will increase calcification of vascular walls leading to arterial stiffness and subsequently to increased SBP. In support of this suggestion the study of Mori and coworkers is important who found a positive association between Fetuin-A level and arterial stiffness. Additionally, Schafer and colleagues have demonstrated that Fetuin-A deficient-mice undergo severe calcification in various organs.

In another study, the low serum levels of Fetuin-A have also been associated with malnutrition, inﬂammation, atherosclerosis with increased cardiovascular and all causes of mortality. This suggests that ISH patients with low level of Fetuin-A are more subjective to cardiovascular diseases. This suggestion also gets support from an earlier study demonstrating that increased SBP is more associated to cardiovascular ailments than diastolic. In addition, the reduction of Fetuin-A, which is a liver-derived glycoprotein, is also found in patients with end stage renal disease. Ketteler et al. have measured Fetuin-A level in several hundred patients and found that patients on dialysis with low serum level of Fetuin-A showed signiﬁcantly poorer survival as compared to those of normal or high-normal level. Additionally, Fetuin-A may have an important anti-inﬂammatory function because it has been shown to limit the production of cytokine by macrophage and protection against tumor necrosis factor, suggesting that patient with low serum level of Fetuin-A such as those with ISH may be subjected to inﬂammatory diseases, atherosclerosis and arterial stiffness.

The mechanism by which the level of Fetuin-A is reduced in ISH is yet to be known. The parameters and findings of present study do not allow to hypothesise on its mechanism, except that Fetuin-A gene has been shown to possess at least four
polymorphisms, indicating that genetic alteration might affect the circulating level of Fetuin-A in ISH.

On the bases of above discussion along with the results of the present study, it is suggested that the reduced level of vascular anti-calcification hormone, Fetuin-A, in ISH patients, indicate its contribution to arterial stiffness and subsequently rise in the SBP in this group of patients.

The present study demonstrates that ET-1 is significantly increased in HTN patients as compared to ISH and normal subject. This result supports the finding of Kohno et al. who found that ET-1 concentrations in the plasma were higher in HTN patients than in borderline hypertensive and normotensive. However, in the present study the serum level of ET-1 in ISH patients is unchanged as compared to normal, suggesting different pathophysiological mechanism involved in ISH (only SBP is elevated) than in the case of HTN, where both the SBP and DBP are elevated.

Up-regulation of ET-1 has been observed in various cardiovascular conditions. In healthy humans, ET-1 increases mean arterial blood pressure, reduces heart rate, cardiac output and stroke volume and causes potent and long lasting vasoconstriction in the pulmonary, renal, myocardial, and skeletal muscle vasculature. ET-1 may also induce indirect vasoconstrictive effects due to the generation of thromboxane-A2, which is another potent vasoconstrictor. Elevated plasma concentrations of ET-1 have been reported in pre-eclampsia, which could explain the elevated level of blood pressure in this group of patients.

ET-1 has also been implicated in inflammatory processes within the vascular wall. ET-1 enhances the expression of adhesion molecules and stimulates aggregation of neutrophils. Hypercholesterolemia is associated with impaired elevated plasma and tissue ET-1 concentrations, which may account for the vasomotor dysfunction under this condition. ET-1 in sub-nanomolar concentrations has been demonstrated to activate macrophages, resulting in the release of pro-inflammatory and chemotactic mediators, including tumour necrosis factor (TNF)-α, interleukin (IL)-1, IL-6 and IL-8, which are of importance in the atherosclerotic process. Therefore, in addition to its role in developing abnormal vascular tone in hypertensive patients as observed in this study, those patients with elevated level of ET-1 are more likely to develop atherosclerosis.

The mechanism by which ET-1 is elevated in hypertensive patients is not totally understood. Several lines of evidence support the hypothesis that Angiotensin-II (Ang-II) stimulates the production and release of ET-1 in HTN. Other studies demonstrated the involvement of oxidative stress in the production of ET-1 in HTN, because antioxidant treatment with the superoxide dismutase mimic or the combination of vitamins C and E reduce Ang II-induced changes in ET-1 expression. Nitric oxide (NO) is known to inhibit the production of ET-1, possibly via inhibiting superoxide. Reduced levels of nitric oxide synthetase (eNOS) in ISH has already been reported which subsequently lead to reduction of NO. This finding suggests that the reduced level of NO might contribute to the up-regulation of ET-1 at least in hypertensive patients. In addition, increased expression of ET-1 gene in blood vessels of certain models of experimental hypertension has also been reported. Considering the potentially important role of ET-1 in the development of vascular dysfunction, conditions with increased inflammatory activity, oxidative stress and vascular tone such as hypertension and atherosclerosis may be of interest to explore the therapeutic benefits of ET-1 inhibition, in larger clinical trials.

CONCLUSION

Low level of the anti-calcification hormones, Fetuin-A, in ISH suggests its possible contribution in the arterial stiffness and therefore increase in the SBP. Increase in endothelin-1, and decrease in testosterone in hypertensive patients suggest their possible involvement in the pathophysiology of this vascular disease. These findings open a new understanding of the pathophysiology of ISH and hypertension and bring hope for better treatment of these disease states. Large trial studies are required based on medication regulating these factors.

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