

Full Length Research Paper

Elevated Plasma Endothelin-1 Levels as a Marker of Vascular Injury in Hypertension and Diabetes

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The aim of the present study was to determine plasma levels of endothelin-1 (ET-1) in normal healthy population, patients with diabetes mellitus (DM) and its correlation with hypertension and nephropathy in DM. A total of 34 patients with DM, that included 19 cases with simple DM without nephropathy and 15 cases with diabetic nephropathy were used in the study. Four of the cases were considered simple DM and seven cases with diabetic nephropathy were also hypertensive. Thirty three healthy controls were included in the study. Endothelin estimation was done by indirect enzyme linked immunosorbent assay (ELISA). In healthy individuals, plasma ET-1 levels ranged from 2.6 to 5.6 pg/ml with a mean level of $XX \pm YY$. The mean value of ET-1 in diabetic nephropathy as well as in non-complicated DM was significantly higher compared to controls. ET-1 was significantly elevated in DM with hypertension compared to than in control group. We conclude from this study that ET-1 is more elevated in DM associated with hypertension and nephropathy than simple DM. The significant findings of the study are that ET-1 is significantly increased in DM whether associated with nephropathy or not and also in DM with hypertension as compared to healthy controls. The increased plasma ET-1 might be a precipitating factor for DM in the predisposed individuals. We recommend plasma ET-1 assessments in DM patients especially in male patients.

Key words: Endothelin, diabetes mellitus, hypertension, endothelial dysfunction.

INTRODUCTION

Endothelin (ET) is a peptide which was discovered by Yanagisawa et al. (1988), he found ET produces contraction of porcine coronary artery and it was more potent than angiotensin II in producing contraction of blood vessels (Yanagisawa et al., 1988).

There are three different isoforms of ET termed ET-1, ET-2 and ET-3. All three isoforms are made up of 21 amino acids. There is homology between ET-1 and ET-2. ET-1 is the major active isoforms expressed in the vasculature (Schiffrin, 2005). ET is formed by cleaving

amino acids from pre-proendothelin (ProETs) by endopeptidase(s) to generate 38 to 39 amino acids peptides which are called as Big ETs. Big ETs are further converted into mature ETs by endothelin converting enzymes which basically are of 2 types ECE-1 and ECE-2. There are four spliced isoform of ECE-1 called as ECE1a, 1b, 1c, 1d (Valdenaire et al., 1999), all are encoded by one gene, but differ by their N terminal amino acid. ECE 1b is an intracellular enzyme (D'Orléans-Juste et al., 2003) whereas other have catalytic domain outside the cells.

ECE1b forms heterodomains with other ECE-1 isoforms and regulate extracellular ECE-1 activity (Muller et al., 2003). ECE-2 is present on smooth muscle cells and

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converts big ET-1 to ET-1 close to ET receptors. ET are produced by cells of many different organs including endothelial cells, vascular smooth muscle cells of arteries and veins, fibroblast, myocytes in heart and several types of cells in kidney, lung, gut, brain, peripheral endocrine tissue and placenta (Kohan, 1997; Resink et al., 1990; Properzi et al., 1995). Contrary to ET-2 and ET-3, ET-1 is also produced by vascular endothelial cells where it is more abundant. ET-1 is also produced by cells of cancer, pancreas, mast cells, various other endothelial epithelial and smooth muscle cell populations (Ergul, 2002).

Expression of ET-1 is stimulated by hypoxia, angiotensin II, nor-epinephrine, vasopressin, F-isoprostane, serotonin while vasodilators such as nitric oxide, prostaglandins E2 and I2, atrial and brain natriuretic factors inhibit ET-1 production and release (Krämer et al., 1994; Rajagopalan et al. 1997; Shreenivas and Suzanne, 2007).

Endothelins are powerful vasoconstrictor peptides and abundantly present in blood vessel, hence it has role in hypertension. Production of ET-1 is increased in the endothelin and the kidney in a salt dependent model of hypertension (ref). ET-1 elicits an inflammatory response by increasing oxidant stress in the vascular wall remodeling and endothelial dysfunction in hypertensive models (Schiffrin, 2005; Villar et al., 2005; Stjernquist, 1998).

Endothelin receptor antagonist lowers the blood pressure in hypertensive patients. In humans there are reports by Krämer et al. (1994); Shreenivas and Suzanne (2007) and Cassone et al. (1996) that ET-1 is elevated in hypertension. Elevated levels have also been found in patients with uremia (Totsune et al., 1989; Koyama et al., 1989). Hypertension is a very common problem in diabetic patients. The aim of the present study was to find out levels of ET-1 in normal healthy population, patients of diabetes mellitus (DM) and its correlation with hypertension and nephropathy in DM.

Recent studies have also shown increased ET-1 production in hypertension and chronic kidney disease (CKD). ET-1 causes vasoconstriction, inflammation and fibrosis thereby promotes hypertension and atherosclerosis in CKD. ET-1 antagonist improves proteinuria in CKD (Kohan, 2010).

Most of the complications of retinopathy, nephropathy and neuropathy are due to disturbed microvascular function, structural and functional changes in the microcirculation present in DM. There is imbalance between endothelial derived vasodilatation and vasoconstrictor substances in DM. ET-1 is potent vasoconstrictor substance and is found increased with microangiopathy in Type II DM. Vascular endothelial dysfunction may precede insulin resistance (Creager et al., 2003; Kalani et al., 2007). Many studies have shown positive correlation between plasma and urinary level of ET-1 in diabetic nephropathy in various stages including glomerular filtration rate (GFR), mesangial cell

expansion, uremia and microalbuminuria (Candido and Allen, 2002; Zanatta et al., 2008).

MATERIALS AND METHODS

A total of 34 patients of Diabetes Mellitus were taken from the outpatient department of Nephrology of Sir Sunderlal hospital, Banaras Hindu University, Varanasi during a period of 3 months. Out of which 19 cases were simple DM without nephropathy and 15 cases were of DM with nephropathy. Four of the simple DM and seven cases of diabetic nephropathy were hypertensive also. Thirty-three healthy controls from staffs and student of the institute were included in the study with no present and past history of hypertension or DM. ET-1 measurements were accomplished by indirect enzyme linked immunosorbent assay (ELISA), (R and D system, U.S.A., supplied by M/S Zentech India Varanasi, India) following the manufactures directions. Briefly, antibody specific to ET-1 was pre-coated on micro plate. Standards, samples, control, conjugates were pipetted into the wells. Any ET-1 present in sample is sandwiched by immobilized antibody and enzyme linked antibody specific to ET-1. After washing substrate is added to develop colour which is stopped by sulphuric acid and the colour is measured spectrophotometrically. Absorbance is inversely proportional to the concentration of ET-1 in the sample.

Statistical analysis

The statistical analysis of the data was done using Student's t test for difference of means on SPSS for windows (Version 16.0) statistical package (SPSS Inc., Chicago, IL) computer statistics program. P values less than 0.05 was taken as a statistically significant difference.

RESULTS

Plasma ET-1 levels in healthy controls varied from 2.6 to 5.6 pg/ml. Mean value of control cases was 4.21 ± 0.84 pg/ml. A trend of decreasing ET-1 was seen with increasing age, though no statistical difference was detected between age groups (Table 1).

ET-1 level was correlated with the sex of the patients. Although there was mild increase in ET-1 in males as compared to females (5.5 ± 2.2 vs. 4.3 ± 0.83 pg/ml) and in DM cases compared to the control group (4.8 ± 1.0 vs. 4.1 ± 0.86 pg/ml), the differences were not statistically significant (Table 2).

ET-1 was measured in the 34 cases of DM of which 15 were with nephropathy while 14 were of simple DM without any complications.

In diabetic nephropathy group 2, (13.33%) patients had ET-1 below 3 pg/ml and the majority 8 (53.33%) patients had ET-1 between 3 to 5 pg/ml. 5 cases (33.33%) had ET-1 values above 5 pg/ml. The mean value of ET-1 in diabetic nephropathy as well as in non complicated DM was significantly higher compared to controls (Table 3). The mean value of ET-1 was higher in the nephropathy group (5.7 ± 2.2 pg/ml) as compared to non-complicated group (4.9 ± 1.2 pg/ml) but the differences were not statistically significant. ET-1 level were also analyzed

Table 1. Showing age wise distribution of endothelin in healthy controls.

| Groups age in years | Total no. of cases | Endothelin (pg/ml) Mean±SD | t | p |
|---------------------|--------------------|----------------------------|---------------|-------|
| (A) 20-30 | 3 | 4.56±0.63 | A vs. B 0.596 | 0.558 |
| (B) 30.1-40 | 18 | 4.24±0.88 | B vs. C 0.463 | 0.647 |
| (C) 40.1-50 | 8 | 4.07±0.78 | C vs. D 0.046 | 0.964 |
| (D) 50.1-60 | 4 | 4.10±1.08 | A vs. C 0.963 | 0.361 |
| Total | 33 | 4.21±0.84 | A vs. D 0.654 | 0.542 |
| | | | B vs. D 0.283 | 0.780 |

P value<0.05 is significant.

Table 2. Showing sex wise distribution of endothelin in diabetes mellitus and healthy controls.

| Groups | Male(A) Mean±SD | Female(B) Mean±SD | PA vs. B |
|-------------------|--------------------|----------------------|----------|
| Diabetes mellitus | 5.45±2.15 | 4.82±1.03 | 0.295 |
| Controls | 4.31±0.834 | 4.06±0.869 | 0.431 |

P value<0.05 is significant.

Table 3. Level of endothelin in diabetes mellitus with and without renal complications and controls.

| Groups (No of cases) | Range of endothelin | | | | | | Mean±SD | P |
|---|---------------------|-------|-----------|-------|-----------|-------|-----------|---------------|
| | < 3 pg/ml | | 3-5 pg/ml | | > 5 pg/ml | | | |
| | No | % | No | % | No | % | | |
| (A) Diabetes mellitus without any renal involvement(19) | 4 | 21.05 | 10 | 52.63 | 5 | 26.31 | 5.15±1.32 | A vs. C 0.003 |
| (B) Diabetic nephropathy(15) | 2 | 13.33 | 8 | 53.33 | 5 | 33.33 | 5.60±2.09 | B vs. C 0.002 |
| (C) controls | 1 | 3 | 27 | 81.81 | 5 | 15.15 | 4.21±0.84 | A vs. B 0.178 |

P value<0.05 is significant.

according to hypertension. Out of 34 cases, 11 had hypertension. In the hypertensive group all patients had ET-1 concentration above 3 pg/ml. There was a significant relationship between ET-1 and hypertension. ET-1 was significantly elevated in DM with hypertension compared to the control group, 4.94±1.42 vs 4.21±0.84 pg/ml respectively (Table 4).

DISCUSSION

In present study of 33 control cases in the age range of between 19 to 50 years, ET-1 concentrations ranged from 2.30 to 5.30 pg/ml. Similarly, Cassone et al. (1996) also reported a similar range between 2.30 ± 5.50 pg/ml but their mean value of ET-1 was lower than us (3.90±1.50 pg/ml).

Reports of ET-1 plasma concentrations in DM vary considerably with positive correlation. In a streptozotocin diabetic rat model, the duration of diabetes appears to determine the direction of changes in plasma ET-1 (Cassone et al., 1996; Totsune et al., 1989; Koyama et al., 1989).

An experiment is conducted on 100 patients of DM and

19 healthy controls, and found that ET-1 was increased more than two folds in DM (1880 ± 50 fmol/l) as compared to controls (540 ± 50 fmol/L) but they did not find any correlation of ET-1 with blood pressure, renal disease, retinopathy, duration of DM and level of fasting blood sugar (Tada et al., 1994). Some studies carried out in type I DM reported raised ET1 (Makino and Kamata 1998; Hopfner et al., 1999). While some groups reported decrease of ET-1 in type I DM (Takahashi et al., 1990; Collier et al., 1992). Similarly, in type II DM there are controversial reports. Some studies, Hoak et al. (1992) and Smulders et al. (1994) reported an increase in ET-1 levels in type II DM while others (Malamitsi-Puchner et al., 1996; Morise et al., 1995) did not find any change from normal controls. Similar to these studies, we also found slightly but significantly elevated ET-1 in DM with nephropathy and DM associated with hypertension as compared to non complicated DM.

Thus, we conclude from this study that ET-1 is more elevated in DM compared to healthy individuals and in DM with hypertension and nephropathy than in simple diabetes mellitus. Additional studies in larger series are suggested in order to investigate the potential role of ET-1 in DM with hypertension and nephropathy.

Table 4. Levels of endothelin in diabetes Mellitus with and without hypertension and controls.

| Groups (No of cases) | Range of endothelin | | | | | | Mean±SD | P |
|--|---------------------|------|-----------|-------|-----------|-------|-----------|---------------|
| | < 3 pg/ml | | 3-5 pg/ml | | > 5 pg/ml | | | |
| | No | % | No | % | No | % | | |
| (A) Diabetes mellitus (23) | 1 | 4.34 | 15 | 65.21 | 7 | 30.43 | 5.27±1.90 | A vs. C 0.005 |
| (B) Diabetic mellitus with hypertension (11) | 1 | 9 | 7 | 63.63 | 3 | 27.27 | 4.94±1.42 | B vs. C 0.046 |
| (C) controls | 1 | 3 | 27 | 81.81 | 5 | 15.15 | 4.21±0.84 | A vs. B 0.508 |

P value<0.05 is significant.

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