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Estrogen Receptor α and β Status in Hypertensive Postmenopausal Women with Co-morbidities

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ABSTRACT

Menopause is accompanied by decline in estrogen levels and a substantial rise in the incidence of hypertension in majority of women. Whether this decline is in accordance with estrogen receptor levels and associated co-morbidities need to be elucidated. Hence estrogen receptor status was assessed among selected hypertensive postmenopausal women with co-morbidities. A total of 12 postmenopausal women were selected and categorised into normotensive postmenopausal women (group-1), hypertensive postmenopausal women (group-2) hypertensive postmenopausal women with diabetes (group-3) and hypertensive postmenopausal women with renal insufficiency (group-4). Levels of estrogen receptor α and estrogen receptor β were assessed in these groups of postmenopausal women. One way ANOVA was performed using SPSS16.0 statistical software. No significant difference was found in estrogen receptor α levels between the groups. However, estrogen receptor β levels were significantly elevated in hypertensive postmenopausal women with renal insufficiency (group-4). The expression of these receptors might depend on the availability of estradiol. ERβ levels were found to increase with co-morbid conditions.

Keywords: *Estradiol, estrogen receptor, hypertension, peripheral blood mononuclear cells, postmenopause*

INTRODUCTION

The prevalence of hypertension rises more steeply in late middle aged women than in men. Endothelial dysfunction is considered to be one of the mechanisms by which estrogen deficiency may result in hypertension. Endothelial dysfunction with reduction in vasodilators modulating vascular tone is found to be associated with diseases including hypertension and atherosclerosis [1]. Estrogens mediate their action via estrogen receptors, Estrogen Receptor α (ERα) and Estrogen Receptor β (ERβ) [2], [3]. ERα and ERβ are the products of separate genes ESR1 and ESR2, present on distinct chromosomes [4]-[8]. Estrogen receptor α has a full-length 66 kDa isoform (ERα66) consisting of 595 amino acids [9]. Estrogen receptor β encodes a 530 amino acid protein [10].

Estrogen modulates vascular endothelial function through estrogen receptor α-mediated genomic and non-genomic mechanisms by eliciting the release of nitric oxide. Nitric oxide is released by the activation of endothelial nitric oxide synthase (eNOS) and through transcriptional activation of eNOS gene. Therefore normal vascular function depends on functional ERα [11]. Estrogen deficit for long duration leads to significant reduction in ERα levels, adversely affecting ERα/eNOS signaling [12]. Genomic estrogen signaling might be required to maintain basic cellular functions, whereas non-genomic signaling pathways may represent mechanisms that allow rapid adaptation of vascular function in response to changes in surrounding environment [13], thereby rapid estrogen-mediated effects may enhance the ability of cell to vigorously encounter pathological alterations, such as vascular inflammation and atherogenesis [14].

Thus, cardiovascular homeostasis is maintained through genomic and non-genomic actions of estrogen, but the individual contribution of these pathways in health and disease *in vivo* still remains to be elucidated. There is considerable evidence for the altered expression and function of classical ER during different stages of atherogenesis [15]. Consequently suggesting significant alterations in non-genomic and genomic estrogen signaling pathways in diseased vessels [16]. Estrogen receptor levels are strongly associated with endothelial-dependent vasodilation and are found to be reduced in postmenopausal women compared to premenopausal women. Maintaining estrogen levels during the menopausal transition may retain ERα expression and functioning, thereby protecting the vascular endothelial health [17].

The fact that estrogens mediate immune response is well documented [18], [19]. Since estrogen mediates its action through its interaction with estrogen receptors, the analysis of estrogen receptor and estrogen receptor expression in peripheral blood lymphocytes and peripheral blood mononuclear cell subsets provide a useful tool in understanding the responsiveness of these cells to estrogens [20], [21].

Estrogen levels decline with menopause. Whether this decline is in accordance with estrogen receptor levels and associated co-morbidities need to be explicated. Studies on estrogen receptor status among hypertensive postmenopausal women are scarce. Hence it is imperative to assess estrogen receptor status in hypertensive postmenopausal women with clinical complications.

MATERIALS AND METHODS

Postmenopausal women who visited KTVR Hospital, Coimbatore, Tamil Nadu were selected for the study. A written informed consent was obtained from the participants. Institutional Ethics Committee clearance (HEC.2011.25) was obtained for the study. Subjects with hypertension, diabetes and renal insufficiency were alone included in the study. Menopause was confirmed by the absence of menstruation for more than two years. 12 subjects were selected and categorised into four groups namely normotensive postmenopausal women (group-1), hypertensive postmenopausal women (group-2) hypertensive postmenopausal women with diabetes (group-3) and hypertensive postmenopausal women with renal insufficiency (group-4) for the assessment of estrogen receptor status. Normotensive postmenopausal women constituted the control group.

Blood was collected in EDTA tubes. Peripheral Blood Mononuclear Cells (PBMC) were isolated from EDTA blood using Ficoll Histopaque-1077. The cells were washed by adding isotonic phosphate buffered saline solution, mixed by gently drawing in and out of a pasteur pipette and centrifuged at 250xg for 10 minutes. The supernatant was aspirated and discarded. The washing steps were repeated, supernatant was discarded and cell pellet was resuspended in 0.5 ml of isotonic phosphate buffered saline solution [22]. Protease inhibitor, phenylmethylsulfonylfluoride (PMSF) and cooled lysis buffer (1% w/v Triton X-100 in phosphate buffered saline (PBS) containing 1mM EDTA) were added to the cooled cell suspension. This was left on ice for 30 minutes, centrifuged at 4^0C and total PBMC extract was prepared by the method of Rosenberg [23]. PBMC extract was added to Sephadex G-100 column (1 x 30cm) equilibrated with TEM buffer. The column was eluted with TEM buffer (10mM Tris, 1mM EDTA, 12mM Monothioglycerol, 0.2mM PMSF) pH 7.6 and fractions were collected [24], [25].

The fractions were subjected to SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE). 3% stacking gels and 12% separating gels were prepared. Sample buffer was added to the eluted protein fractions. The protein fractions were denatured following heating in a boiling water bath for 3-5 minutes. This was cooled and pulse centrifuged. The protein fractions were loaded into the gels. The gels were run at a constant voltage of 60V. When the tracking dye reached the end of the gel, the run was terminated. SDS-PAGE was performed by the method of Laemmli [26]. The electrophoresed polyacylamide gels were transferred to the gel staining box and were silver stained [27]. The fractions that produced the specific bands corresponding to estrogen receptors α and β were further subjected to HPLC analysis for the quantification of isolated estrogen receptors. The column used was phenomenex reverse phase C-18 with 25 cm x 2.5 mm column dimension. The filtered sample (10 µl) was injected to the automatic injector using a microsyringe (1-20 µl, Shimadzu). The mobile phase was in isocratic mode. The flow rate was maintained to 1.3 ml/min with a column temperature of 25^0C. The class VP integration software was used for the data analysis.

One way ANOVA was performed using SPSS16.0 statistical software for windows to compare

percentage fraction of estrogen receptor α and estrogen receptor β among the four groups of participants.

RESULT AND DISCUSSION

Peripheral blood mononuclear cells (PBMC) were isolated using Ficoll Histopaque-1077. PBMC were lysed with lysis buffer supplemented with protease inhibitor and total PBMC extract was prepared. The cell extract was subjected to Sephadex G-100 column chromatography and was eluted with TEM buffer. The flow rate was maintained at 4.2ml/hr. Fractions were collected at definite intervals and subjected to SDS-Polyacrylamide gel electrophoresis. The electrophoresed polyacylamide gels were silver stained and bands were identified as 66 kDa ERα and 56 kDa ERβ (Figure 1).

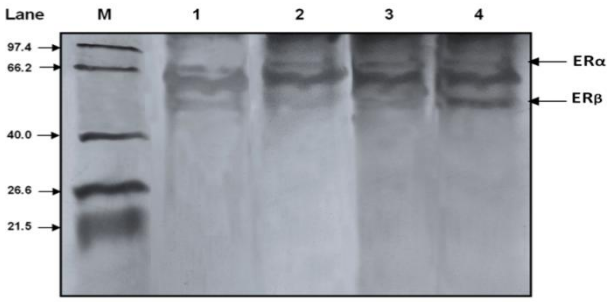


Figure 1 Bands showing Estrogen Receptor α and Estrogen Receptor β by SDS-PAGE

Lane M - Protein marker

Lane 1 - normotensive postmenopausal women as control (Group 1)

Lane 2 - hypertensive postmenopausal women (Group 2)

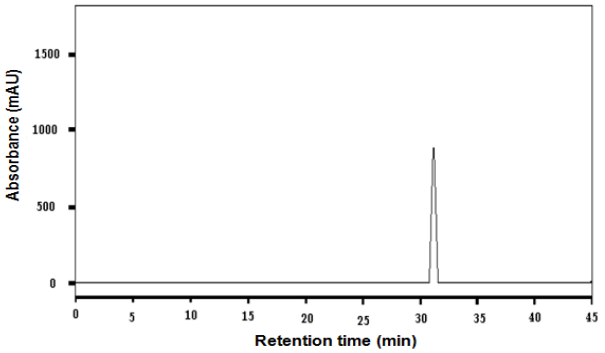
Lane 3 - hypertensive postmenopausal women with diabetes (Group 3)

Lane 4 - hypertensive postmenopausal women with renal insufficiency (Group 4)

The fractions that produced the specific bands corresponding to ERα and β were subjected to HPLC analysis for the quantification of the isolated estrogen receptors (Figure 2 – 7).

Pierdominici *et al* also perceived positive ERα and ERβ signals in lymphocyte subsets, namely $CD4^+$ and $CD8^+$ T lymphocytes, $CD19^+$ B lymphocytes and $CD3^-CD56^+$ NK cells [20]. 66 kDa ERα and 56 kDa ERβ were identified in soleus and extensor digitorum longus muscles and adipose tissue by SDS-PAGE followed by western blot analysis in female rats fed a high fat diet [28]. SDS-PAGE and western blot analysis recognized ERα band with molecular size of 66 kDa in cell lysates from stable NPE3-3/MCF-7 and pcDNA/MCF-7 clones [29]. The present study reports are in accordance with these statements.

Figure 2 HPLC chromatogram of Estrogen Receptor α standard



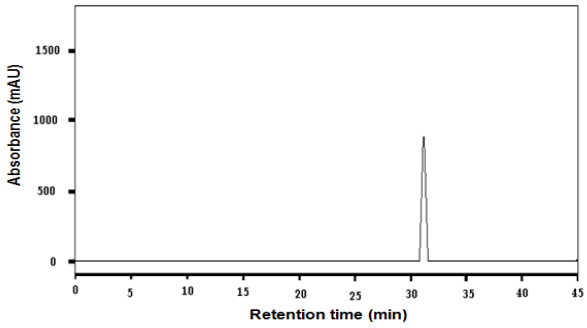


Figure 3 HPLC chromatogram of Estrogen Receptor β standard

Figure 4 HPLC chromatogram of Estrogen Receptor α and β in Normotensive Postmenopausal Women

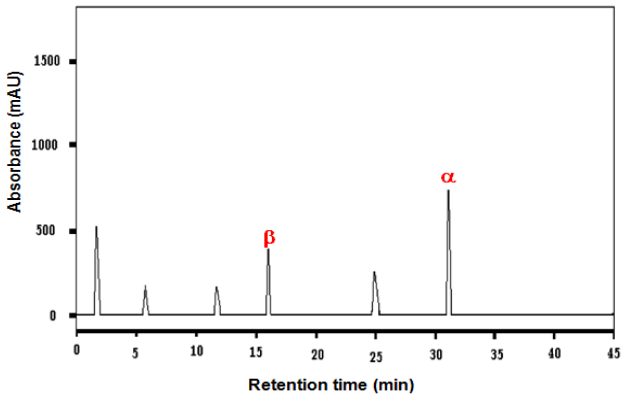


Figure 5 HPLC chromatogram of Estrogen Receptor α and β in Hypertensive Postmenopausal Women

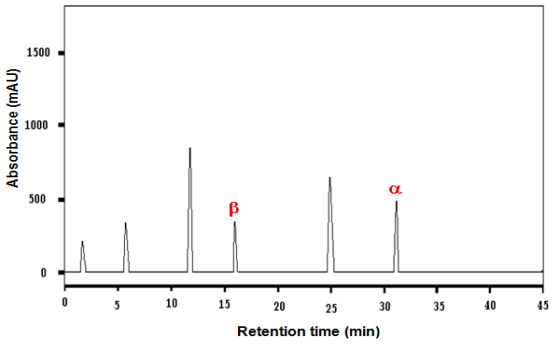


Figure 6 HPLC chromatogram of Estrogen Receptor α and β in Hypertensive Postmenopausal Women with Diabetes

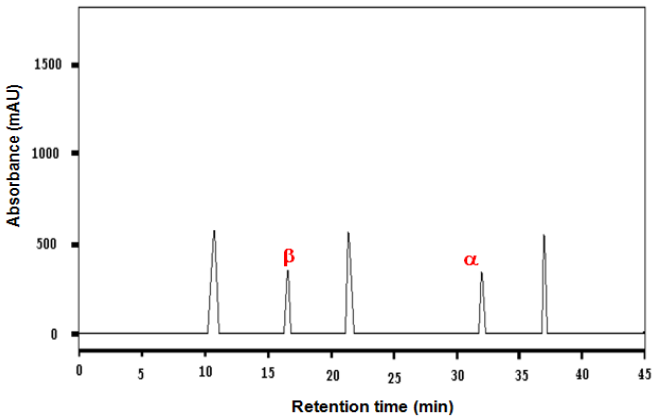
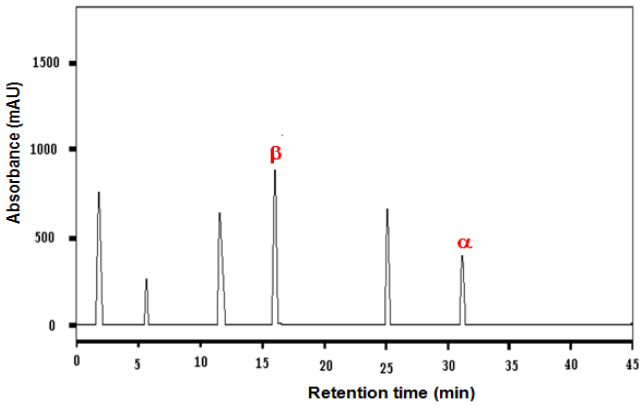


Figure 7 HPLC chromatogram of Estrogen Receptor α and β in Hypertensive Postmenopausal Women with Renal Insufficiency



The percentage fraction of the two estrogen receptors α and β are shown in Table1.

Table 1 Estrogen receptor status in normotensive and hypertensive postmenopausal women with and without diabetic and renal abnormalities

Groups	Estrogen receptor α (ERα) (%)	Estrogen receptor β (ERβ) (%)
Normotensive postmenopausal women (a)	29.85 ± 10.45	31.45 ± 2.53
Hypertensive postmenopausal women (b)	23.30 ± 1.29	37.71 ± 3.14
Hypertensive postmenopausal women with diabetes (c)	19.18 ± 3.30	48.10 ± 11.08[a]
Hypertensive postmenopausal women with renal insufficiency (d)	23.72 ± 8.74	73.29 ± 4.38[abc]

Values are Mean ± SD

The superscript alphabets of a specific group mean denote the statistically significant difference of that group at 5% level Estrogen receptor α (ERα) levels did not exhibit any significant difference between the groups. Estrogen receptor β (ERβ) levels were significantly elevated in hypertensive

postmenopausal women with renal insufficiency (group-4) compared to normotensive postmenopausal women (group-1), hypertensive postmenopausal women (group-2) and hypertensive postmenopausal women with diabetes (group-3). There was a significant increase in ERβ levels in hypertensive postmenopausal women with diabetes (group-3) compared to normotensive postmenopausal women (group-1).

In the present study, ERα levels were decreased and ERβ levels were significantly increased in the three experimental groups compared to normotensive postmenopausal women (group-1) (Figure 8). This suggests that the expression of these receptors might depend on the availability of estradiol. Sitges *et al* opined that ERα and ERβ mRNA expression was enhanced in arterial vessels of postmenopausal women with coronary artery disease after a short-term treatment with transdermal estradiol as compared to those who did not receive the estradiol treatment [30].

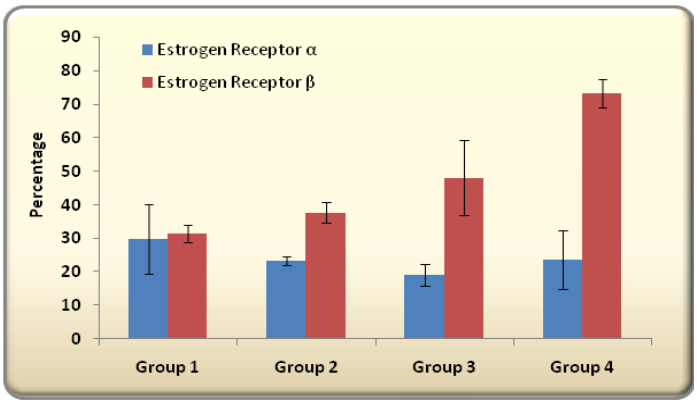


Figure 8 Estrogen Receptor α and Estrogen Receptor β levels

Group 1 - normotensive postmenopausal women as control

Group 2 - hypertensive postmenopausal women

Group 3 - hypertensive postmenopausal women with diabetes

Group 4 - hypertensive postmenopausal women with renal insufficiency

Another study reported that in the skin samples of obese premenopausal women, ERα and ERβ were decreased than their non-obese counterparts. On the other hand, in obese postmenopausal women the levels of ERα and ERβ were elevated compared to those of non-obese postmenopausal women. They also stated a reduction in the levels of ERα and ERβ among postmenopausal women compared to premenopausal women [31]. In both postmenopausal and premenopausal women $CD4^+$ T cells expressed increased levels of ERα compared to ERβ. But ERβ levels were higher in B cells than that of ERα. $CD8^+$ T cells expressed very low levels of ERα and ERβ compared to other peripheral blood mononuclear cell subsets. Monocytes of postmenopausal women expressed significantly higher levels of ERα than that of premenopausal women. There was no difference in ERβ levels in the monocytes of the two groups [21].

In the present study, ERβ levels were found to increase with co-morbid conditions. The possible reason for elevated levels of ERβ with co-morbidities might be due to the oppose action of ERβ to ERα. The imbalance in the expression of ERα and ERβ levels might be the root cause of several estradiol-dependent diseases. The peculiar feature of ERβ is that it opposes the biological action of ERα, suggesting a yin-yang' relation between the two receptors [32]-[37]. Lamote *et al.* revealed the expression of estrogen receptor β in neutrophils of bovine blood [38]. ERα suppression in

the ventromedial nucleus of hypothalamus (VMN) of adult female mice lead to the development of metabolic syndrome characterised by increased body weight, physical inactivity, excess visceral fat, hyperphagia, glucose intolerance, low basal metabolic rate and impaired thermogenic response to feeding [39]. In diabetes, ERβ overexpression through its antiangiogenic effect contributed to impaired wound healing. The loss of function of ERβ promoted wound healing and might be due to an exclusive ERα-mediated signaling that contributed to angiogenesis [40]. The protective effects of E2 can be retained through specific activation of ERαAF-2. Thus help in the development of beneficial therapeutic strategies to prevent obesity, metabolic syndrome and type 2 diabetes without raising the possible risk of E2-dependent malignancies in postmenopausal women [41], [42].

CONCLUSION

The SDS-PAGE run polyacylamide gels were silver stained and bands were detected as 66 kDa ERα and 56 kDa ERβ. There was no significant difference in estrogen receptor α (ERα) levels between the groups. However, estrogen receptor β (ERβ) levels were significantly elevated in hypertensive postmenopausal women with renal insufficiency (group-4) compared to the other three groups. The expression of these receptors might depend on the availability of estradiol. ERβ levels were found to increase with co-morbid conditions. The imbalance in the expression of ERα and ERβ levels might result in ERβ hyperactivity which might inturn oppose the activity of ERα.

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