Hypertension is characterized by systolic blood pressure (BP) of 140 mmHg or higher or a diastolic BP of 90 mmHg or higher at the age of 20 years, and 160/95 mmHg at the age of 50 year [3-5]. The blood pressure is the force applied against the walls of the arteries as the heart pumps blood through the body. The pressure is determined by the force and amount of blood pumped and the size and flexibility of the arteries [1].

There are two stages of hypertension. Stage-I comprises systolic BP between 140-159 mmHg and diastolic BP between 90-99 mmHg; whereas stage-II with systolic BP ≥ 160 mmHg and diastolic ≥ 100 mmHg [4]. Hypertension results from two major factors that may present independently or together firstly pumping of blood with excessive force by the heart and secondly by narrowing of the arterioles resulting in more pressure against the vessel’s walls exerted by blood flow. Risk factors of hypertension can be seen in blood pressure (BP) as low as 115/75 mmHg and will begin to double in risk for every 20/10 mmHg increase [6]. High blood pressure causes extra burden on heart, making it so bigger that the oxygen flow is disrupted leading to heart attack [7]. Hypertension can also cause cardiomyopathy [8]. Hypertension is a leading cause of stroke and coronary heart disease, and is a major contributor to the onset and progression of chronic heart failure [7, 9, 10].

In females blood pressure also vary during different phases of menstrual cycle. This variation of blood pressure is due to the effect of ovarian hormones on cardiovascular function. Hormonal changes follow a non-linear trend throughout the menstrual cycle and thus have unpredicted effect on blood pressure regulation. Women in reproductive age have relatively less chance of hypertension and coronary artery disease [11]. Never or curtailed lactation increases the risk of maternal hypertension [12,13].

Many different drugs can temporarily elevate the blood pressure or worsen the existing high blood pressure, including corticosteroids introduced orally or intravenously. Nonsteroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen (Motrin), naproxen (Aleve), acetaminophen, and aspirin also elevate blood pressure [14,15]. Oral contraceptives (birth control...
pills) increase the risk of hypertension [16]. Estrogen inhibits sympathetic nervous activity and in this way protects against elevated arterial pressure in premenopausal women [17]. High plasma level of endogenous estradiol is a predictor of ischemic arterial disease in older postmenopausal women [18]. Hypertensive disorders of pregnancy are one of the main causes of maternal and pre-natal death and morbidity in the world. Androgens play role in mediating the hypertension in young women with Polycystic ovary syndrome [19]. Low serum testosterone levels are associated with multiple risk factors for hypertension and cardiovascular disease (CVD) and cardiovascular mortality in men [20-25]. Low level of free testosterone results in elevated blood pressure in men with increasing age [26].

METHODS
Estradiol Level
Serum samples were analyzed for activity of estradiol by using the Bio Check Estradiol(E2)Enzyme Immunoassay Test Kit Catalog Number: BC-111

All reagents were brought to room temperature (18-25°C) before use.

Procedure
Secured the desired number of coated wells in the holder. Dispensed 25µL of standards, specimen and controls into the appropriate wells. Dispensed 100µL of Estradiol-HRP Conjugate Reagent into each well. Dispensed 50µL of rabbit anti-Estradiol(E2) reagent to each well. Thoroughly mixed for 30 seconds as it was very important to mix them completely. Incubate at room temperature (18-25°C) for 90 minutes. Rinsed and flicked the microwells 5 times with distilled or deionized water. Striked the wells sharply onto absorbent paper or paper towels to remove all residual water droplets. Dispensed 100 µL of TMB Reagent into each well and gently mixed for 5 seconds. Incubate at room temperature (18-25°C) for 20 minutes. Then stopped the reaction by adding 100 µL of stop solution to each well. Gently mixed 30 seconds and made sure that all the blue colour changed to yellow colour completely. Read the absorbance at 450 nm with a microtiter well reader within 15 minutes [27].

Testosterone Level
Serum samples were analyzed for the activity of testosterone by using the Bio Check Testosterone Enzyme Immunoassay Test Kit Catalog Number: BC-115.

All reagents were brought to room temperature (18-25°C) before use. All reagents were mixed by gentle inversion or swirling prior to use.

PROCEDURE
Secured the desired number of coated wells in the holder. Dispensed 10µL of standards, specimen and controls into the appropriate wells. Dispensed 100µL of Testosterone-HRP Conjugate Reagent into each well. Dispensed 50µL of rabbit anti-Testosterone reagent to each well. Thoroughly mixed for 30 seconds as it was very important to mix them completely. Incubate at room temperature (37°C) for 90 minutes. Rinsed and flicked the microwells 5 times with distilled or deionized water. Striked the wells sharply onto absorbent paper or paper towels to remove all residual water droplets. Dispensed 100 µL of TMB Reagent into each well and gently mixed for 5 seconds. Incubate at room temperature (18-25°C) for 20 minutes. Then stopped the reaction by adding 100 µL of stop solution to each well. Gently mixed 30 seconds and made sure that all the blue colour changed to yellow colour completely. Read the absorbance at 450 nm with a microtiter well reader within 15 minutes [28].

Statistical analysis
Statistical analysis was carried out by using SPSS 20th version and graph Instat 3. One-sample t-test was applied for comparison with the reference range, on data regarding hormones. Differences were considered statistically significant at P<0.05.

RESULTS
Evaluation of estradiol level in post-menopausal cardiovascular hypertensive patients
The average estradiol level (61.38pg/ml) was much higher in post-menopausal hypertensive females than the reference value <18 pg/ml. The lower and upper limits of estradiol in cardiovascular females remained as 5.45 pg/ml and 90pg/ml respectively. The difference between the two means was 0.89286 (Table 1).

Table 1: Evaluation of estradiol level in post-menopausal cardiovascular hypertensive patients

<table>
<thead>
<tr>
<th>Test Value = &lt;18 pg/ml</th>
<th>Estradiol in post-menopausal female</th>
</tr>
</thead>
<tbody>
<tr>
<td>t</td>
<td>df</td>
</tr>
<tr>
<td>15.000</td>
<td>27</td>
</tr>
</tbody>
</table>

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Evaluation of estradiol level in ovulating female cardiovascular hypertensive patients

A normal level of estradiol was observed in cardiovascular hypertensive females that were in ovulating phase. The average estradiol level in ovulating females was 51.01 pg/ml. The testing value was 30-100 pg/ml. The two tailed significance value was 0.351. The degree of freedom was 7 (Table.2)

<table>
<thead>
<tr>
<th>Test Value</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Estradiol in ovulating female</td>
<td>30-100 pg/ml</td>
</tr>
</tbody>
</table>

Table.2: Evaluation of estradiol level in ovulating female cardiovascular hypertensive patients

<table>
<thead>
<tr>
<th>t</th>
<th>df</th>
<th>Sig.(2-tailed)</th>
<th>Mean Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.000</td>
<td>7</td>
<td>.351</td>
<td>.25000</td>
</tr>
</tbody>
</table>

Estradiol in cardiovascular hypertensive males

Results obtained after one sample t-test for this data showed that the average estradiol level 51.545 pg/ml in males was higher than the testing value by 10-50 pg/ml. The two tailed significance value was 0.04. The difference between the two means was 0.41176. The degree of freedom was 16 and the t-static value was 3.347. The higher limit of estradiol remained as 100 pg/ml and the lower limit was 30 pg/ml (Table.3).

<table>
<thead>
<tr>
<th>Test Value</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Estradiol in males</td>
<td>10-50 pg/ml</td>
</tr>
</tbody>
</table>

Table.3: Estradiol in cardiovascular hypertensive males

<table>
<thead>
<tr>
<th>t</th>
<th>df</th>
<th>Sig.(2-tailed)</th>
<th>Mean Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.347</td>
<td>16</td>
<td>.004</td>
<td>.41176</td>
</tr>
</tbody>
</table>
Evaluation of testosterone level in post-menopausal cardiovascular hypertensive females

The average testosterone 4.509 ng/ml in post-menopausal females was higher than the testing value by 0.08-0.35 ng/ml. The difference between the two means was 1.52000. The degree of freedom was 24. The t-static value was 14.905.

Table 4: Evaluation of testosterone level in post-menopausal cardiovascular hypertensive females

<table>
<thead>
<tr>
<th>Testosterone in post-menopausal female</th>
<th>t</th>
<th>df</th>
<th>Sig.(2-tailed)</th>
<th>Mean Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>14.905</td>
<td>24</td>
<td>.000</td>
<td>1.52000</td>
</tr>
</tbody>
</table>

Evaluation of testosterone level in ovulating cardiovascular hypertensive females

Higher level 4 ng/ml of testosterone was observed in cardiovascular hypertensive females that were in ovulating phase. For this output the testing value was 0.2-0.8 ng/ml and the t-static value was 8.881 with lower limit of 1.5 ng/ml and higher of 6.5 ng/ml. The difference between the two means was 1.62500. The degree of freedom was 7.
Table-5: Evaluation of testosterone level in ovulating cardiovascular hypertensive females

<table>
<thead>
<tr>
<th>Testosterone in ovulating female</th>
<th>Test Value = 0.2-0.8 ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>t</td>
<td>df</td>
</tr>
<tr>
<td>Mean Difference</td>
<td>Sig.(2-tailed)</td>
</tr>
<tr>
<td>8.881</td>
<td>7</td>
</tr>
</tbody>
</table>

Fig-5: Evaluation of testosterone level in ovulating cardiovascular hypertensive females

Evaluation of testosterone level in cardiovascular hypertensive males

For testosterone a significant difference was found in males with two tailed significance value of 0.020. The testing value was 3.0-10.0 ng/ml. 2.582 was the t-static value. The difference between the two means was 0.58824. The degree of freedom was 16.

Table-6: Evaluation of testosterone level in cardiovascular hypertensive males

<table>
<thead>
<tr>
<th>Testosterone in males</th>
<th>Test Value =3.0-10.0 ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>t</td>
<td>df</td>
</tr>
<tr>
<td>Mean Difference</td>
<td>Sig.(2-tailed)</td>
</tr>
<tr>
<td>2.582</td>
<td>16</td>
</tr>
</tbody>
</table>

Fig-6: Evaluation of testosterone level in cardiovascular hypertensive males

DISCUSSION

We observed that cardiovascular females suffer more with hypertension than cardiovascular males. Prevalence of hypertension among females is 66% while 34% in males Kokiwar et al. [29] also found the same pattern of prevalence of hypertension. Hypertension is more commonly observed in post-menopausal women. The present finding is compatible with Alberto et al. [30] and Barton and Meyer.17 Changes in the level of reproductive hormones viz estradiol and testosterone was observed in cardiovascular hypertensive patients. Post-menopausal

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cardiovascular hypertensive females showed higher concentration of estradiol in their blood serum than the normal. The same finding was also given by Scarabin-Carré et al. [18] Statistically there was no difference in the concentration of estradiol in cardiovascular hypertensive females that were in ovulating phase, than normal value. In male cardiovascular hypertensive patients statistically significant difference was observed with higher level of 100 pg/ml. Testosterone was also found in higher concentration in post-menopausal and the ovulating cardiovascular hypertensive females. In hypertensive males statistically significant difference was observed with lower value of 1.5 ng/ml.

Disclosures: “None”.

REFERENCES
19. Chen, M., Yang, W., Yang, J., Chen, C., B.Ho, and Yang, Y. (2007). Relationship between androgen levels and blood pressure in young women with polycystic ovary syndrome. Hypertension, 49(6), 1442-1447.


