EFFECTS OF ANASTROZOLE ADMINISTRATION ON SERUM ESTROGEN LEVEL OF FEMALE RABBITS

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ABSTRACT

Objective: To assess the effects of anastrozole use as hormonal treatment of breast cancer on the serum estrogen level in an animal model.

Study Design: Laboratory based randomized controlled trial.

Place and Duration of Study: Department of Anatomy, Army Medical College, Rawalpindi in collaboration with National Institute of Health, Islamabad, 7 months from Jun 2012 to Nov 2012.

Material and Methods: Thirty adult female rabbits (New Zealand) average weight 1.2 to 2 kg and age between 6 months to 2 years were selected. Ten were kept in control group A and 10 were kept in experimental groups B and C each. Group B was given anastrozole in the dose parallel to normal human dose and group C was given a dose 10 times higher than the normal dose for six months. After the completion of the study blood estrogen levels were taken to evaluate serum estrogen level of the groups. The results were compared among the groups for statistical significance by using SPSS version 21.

Result: After maintaining a steady dose of anastrozole in both experimental groups for six months, blood samples were taken to calculate the mean serum level of estrogen in each group the normal level was 186.1 ± 63.59 pmol/L in group A and 116.4 ± 48.58 pmol/L and 108.2 ± 20.40 pmol/L in experimental groups B and C respectively. The *p*-value was 0.008 which was statistically significant. The *p*-value between the control and experimental group C was 0.003 which is also highly significant. The comparison of the serum estrogen levels of both the experimental groups B and C was found to be statistically insignificant.

Conclusion: Adminstration of normal dose of anastrazole decreases serum estrogen levels. This decrease is significant statistically. Increasing the dose of anastrozole decreases the serum estrogen levels but this decrease is not significant statistically.

Keywords: Anastrozole, Aromatase inhibitors, Breast cancer, Serum estrogen level.

INTRODUCTION

Breast cancer is the leading cause of death among women worldwide[1]. It is high in developed countries on account of early menarche, late child birth, fewer pregnancies, hormonal contraceptives, use of hormonal therapy for menopausal women and increased detection through mammography[2]. The frequency of breast cancer is also increasing in developing countries due to change in the life styles, late child bearing, having fewer children, consumption of high calorie food, physical inactivity and obesity[3]. In Pakistan, although a cancer registry program does not exist at a national level, studies based on data collected from different institutions and regions indicate that breast cancer is very common in this country.

At the end of the nineteenth century, it was first shown that oophorectomy caused regression of advanced breast cancer. Estrogen deprivation was employed after that as a chemopreventive measure, later on restricted mostly to receptor positive cancers[4]. Estrogen synthesis inhibitors were tried to block estrogen biosynthesis for use at therapeutic level. Estrogen synthesis by the ovaries can be prevented by the continual administration of gonadotrophin releasing hormone (GnRH) agonists; it is not, however, possible to block its synthesis from adrenal androgens. The use of aminoglutethimide is

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limited because of its lack of selectivity though it greatly inhibits aromatase activity[5]. The locally produced estrogens in the cancer tissue play a significant role in breast cancer disease progression along with the circulating estrogens. New drugs are being manufactured to selectively block the production of estrogens at all tissue levels. The non-steroidal or type-II agents e.g. exemestane, anastrozole, letrozole and verozole are presently being used for the treatment of breast cancer as first-line or as second-line drugs after tamoxifen[6]. They are highly efficacious and actually superior to tamoxifen in some adjuvant settings[7] but unlike tamoxifen, they do not increase the threat of uterine cancer or venous thromboembolism[8].

Anastrozole is responsible for the synthesis of estrogens from androgenic substrates and is highly expressed in the placenta[9]. It markedly reduces the serum concentrations of estrogen metabolites, namely estrone, estradiol and estronesulphate. The plasma levels fall by 81-87% for the estrone concentration, about 84-85% for estradiol and by 94% for plasma estronesulphate[10]. This drug dramatically reduces the circulating as well as the local levels of estrogens[5].

The present investigations are, therefore, designed to see the effects of anastrozole on the serum estrogen levels. Increased estrogen levels in obese women taking anastrozole treatment reduce the effectiveness of anastrozole[11].

MATERIAL AND METHODS

The study was conducted in the Department of Anatomy, Army Medical College, Rawalpindi in association with National Institute of Health (NIH), Islamabad. The study was conducted with the approval of ethical committee on animal experiments, of Army Medical College Rawalpindi. Duration of study was six months from Jun 2012 to Nov 2012. The study design was laboratory based randomized controlled trial. Thirty adult female New Zealand white rabbits weighing 1.2-2 kg were used for the experiment. Healthy, active, non-pregnant female animals were taken. They were kept at room temperature for about 7 months in separate cages and were given standard animal house diet.

The animals were randomly divided in to three groups. Group A was the control group comprising of 10 animals. Group B included 10 rabbits that received anastrozole orally at a dose of 0.02 mg/kg/day which is normal dose for 6 months. Group C included 10 rabbits and was administered anastrozole in ten times the normal dose orally. After completion of study, the animals were anaesthetized by giving chloroform inhalation in a glass jar.

Statistical Analysis

The data was entered and analyzed by using SPSS version 21. Independent sample t-tests were used to assess the significance of the results between the control and experimental groups. Results were considered significant at $p<0.05$.

RESULTS

After maintaining a steady dose of anastrozole in both experimental groups for six months, blood samples were taken to calculate the mean level of serum estrogen of the groups which was 186.1 ± 63.59 pmol/L in group A and 116.4 ± 48.58 pmol/L and 108.2 ± 20.40 pmol/L in experimental groups B and C respectively. The *p*-value was 0.008 between groups A and B which was highly significant (table). The *p*-value between the control and experimental group C was 0.003 which is also highly significant. The comparison of the serum estrogen levels of both the experimental group B and C was found to be statistically insignificant.

DISCUSSION

The purpose of the study was to understand the effects of orally administered anastrozole on the serum estrogen levels of adult female rabbits. Alot of work has been done on aromatase inhibition and is presently recommended as treatment of choice for postmenopausal women with hormone-sensitive breast cancer[12]. Anastrozole and letrozole are third-generation nonsteroidal aromatase inhibitors[13] that have

been formerly shown to be extremely effective, causing great decrease in blood estrogen levels in postmenopausal women[14]. These two drugs are third-generation, effective, well-tolerated, nonsteroidal aromatase inhibitors used in the treatment of breast cancer. Past studies have compared the ability of these two drugs to reduce aromatization, and a number of in vitro studies have indicated that letrozole is the more potent aromatase inhibitor compared with anastrozole.

Patients with estrogen and progesterone receptor positive breast cancer, the progression of disease process could be controlled by modifying the effects of these hormones. This aim can be achieved either by blocking the hormonal receptors or by decreasing the overall hormonal level in the body[15]. It is estimated that two-thirds of the invasive breast cancers will be positive for hormone receptors and will require endocrine treatment. Approximately three forth of breast cancers are estrogen receptor alpha (ERα) positive and are termed ER-positive. ERα, a member of the steroid hormone receptor family, arbitrates the effects of estrogens actions that initiates proliferation and survival of ER-positive breast cancer cells.

Hormone therapies have been established which inhibit ER signaling including Selective Estrogen Receptor Modulators (SERM) such as tamoxifen, selective estrogen receptor down-regulators (SERD) such as fulvestrant, and aromatase inhibitors such as letrozole, which dereases estrogen synthesis. However, many patients with ER-positive cancers do not respond to ER therapy. They respond initially sometimes but develop progressive disease within 1 to 2 years due to the development of resistance[16]. Identification of new mediators of ER-regulated effects could yield critical improvements in the treatment of ER-positive breast cancers.

After menopause the sole production of the residual estrogen is from nonglandular sources, in particular from subcutaneous fat. Thus, the amount of adipose tissue present in body is a determinant of the peripheral aromatase activity and plasma estrogen levels in postmenopausal women. The plasma estradiol levels drop from about 110pg/ml (400 pmol per liter) to low levels of around 7pg/ml (25 pmol per liter) after menopause. The level of estradiol in breast-cancer tissue is roughly 10 times the concentration present in the plasma in the postmenopausal women, perhaps because of the presence of intratumor aromatase both in the stroma and parenchymal cells of the carcinomatous tissue[17].

The aromatase inhibitors are the new expansion in the hormonal treatment of breast cancer. Anastrozole selectively aims the aromatase enzyme in tissues and lowers the serum estrogen levels. In postmenopausal women with breast cancer, it is now the first line of endocrine treatment preferred over the previously used tamoxifen[18].

Side effects of aromatase inhibitors resemble those of tamoxifen side effects[19] which include vasodilatation, sweating, obesity, dryness of vaginal mucosa, vulvovaginitis, leucorrhea, urinary tract infection, osteoporosis, osteopenia, arthritis and arthralgia, bone pain, pharyngitis,

Table: Post-HocTukey test showing comparison of serum estrogen levels among groups.

Comparison	Mean ± S.D (pmol/L)	*p*-value
Control group A	186.1 ± 63.59	0.008
Experimental group B	116.4 ± 48.58	
Experimental group B	116.4 ± 48.58	0.922
Experimental group C	108.2 ± 20.40	
Control group A	186.1 ± 63.59	0.003
Experimental group C	108.2 ± 20.40	

p-value <0.05 was considered significant.

paraesthesia, anxiety, depression, reduced intellectual function, mood swings, headache, rash, hypertension, insomnia, back pain, lymphoedema, peripheral edema, cold sweats, dizziness, gastrointestinal disorders like nausea, dyspepsia, abdominal pain, constipation, diarrhea, asthenia, fracture, hypercholesteremia, infections, dyspnea, coughing, chest pain, flu syndrome[20].

CONCLUSION

Adminstration of normal dose of anastrazole decrease serum estrogen levels. This decrease is significant statistically. Increasing the dose of anastrozole decreases the serum estrogen levels but this decrease is not significant statistically.

CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any authors.

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