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Combination of Estriol and Vitamin D assists in improving stress incontinence stage

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Abstract

In world population, almost one in every group of five female suffers from inability to retain urine. This loss of urine occurs due to various reasons but main reasons is the age factor, after crossing fifty this conditions comes to prevail as women are already experiencing pre- and post menopause stage. We selected 120 female mice of age 11-25 months old for the study. We evaluated the level of vitamin D in all of them by vitamin D ELISA assay kit. 40 mice were clubbed together as they were suffering from stage 1 and other 40 were suffering from stage 2. All of them were given the dose of estriol (0.005 mg) together vitamin D at (10 I.U/kg) i.p 3 times a week for a period of 6 weeks. More than one-third of mice are now able to retain urine in both the category. In I category where mice were suffering from stage 1 stress incontinence. Urine loss quantity was reduced 35-50% while coughing and sneezing. For II category 25% remarkable decrease. Therefore, mice in our study being incapable of retaining urine improved their body conditions by using a combination of i.p administered estriol and high dosed vitamin D.

Key-words: Stress incontinence, estriol, vitamin D, elisa assay kit, pad test

Introduction

In world population, almost one in every group of five female suffers from inability to retain urine. This loss of urine occurs due to various reasons but main reasons is the age factor, after crossing fifty this conditions comes to prevail as women are already experiencing pre and post menopause stage accordingly. Unprovoked leakage of urine due to slight increase in intra-bladder pressure on movement or physical actions or on sneezing or coughing is known as medical condition of stress urinary incontinence, this definition is given by the International Continence Society and International Urogynecological Association (Jiang and Damaser, 2012). Other causes are due to estrogen deficiency, weak tissue and inability to absorb vitamin D (Uebbing et al., 2016). Stress incontinence is caused due to multiple pregnancy and child birth especially vaginal deliveries, at pregnancy time stretching of pelvic and sphincter muscle occurs and because of stretching they become weak. Its effect is very well experienced in later age especially during and after menopause. It differs from urge incontinence, which is the unintentional loss of urine caused by contraction of bladder, conventionally related with a sense of urgency to urinate.

Multifarious risk factors related with stress urinary incontinence are bulkiness of body, cravings to smoke more, surgical trauma. Nevertheless, absolute pathophysiology is still not clear.

Nowadays, each second women is undergoing from the concomitant symptoms of vitamin D lack (Spitz, 2014; Reichrath, 2014). With ascending age, the skin's capability of producing vitamin D decreases., lifestyle changes and promotion of sedentary life are one of the main causes to be blamed. Accumulation of vitamin D through diet (egg and fish etc) is not sufficient and for elderly they metabolize very slowly and in less amount (Ross et al., 2011). Samuel with his colleague has suggested that vitamin D deficiency may contribute to pelvic floor disorders like stress urinary incontinence. As it is a very known fact that vitamin D is very important for the entire bone and metabolism of the connective tissue and just for this reason we included the estriol (E₃-the third estrogen) in the study. Estriol increases the absorption of vitamin D which was earlier poorly absorbed in pre and post menopause stage.

Material and Methods

Animals

Swiss Albino mice weighing between 25-35g were used. They were 11-25 months old female mice only, since this study observes menopause mice. We selected 80+40 female mice, in 80 breeding stage has passed and they are suffering from stress

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incontinence whereas 40 is taken as control group (consist of normal and toxic control, it consists 10 each in two groups for both the category). They were housed in polypropylene cages and maintained at 25-30 °C and 50% relative humidity in a natural light/dark cycle. Animals were given food and water ad libitum. Institutional Animal Ethics Committee (IAEC) of Pinnacle Biomedical Research Institute (PBRI), Bhopal (Reg. No. 1824/PO/ERe/S/15/CPCSEA) had approved the study and study approval reference number is PBRI/IAEC/PN-16026.

Drugs

Estriol and 25-hydroxy-Vitamin D were purchased from Sigma, USA. Estriol was dissolved in Dimethyl sulfoxide (DMSO) and administered in dose of 0.005 mg/kg i.p. 25-hydroxy-Vitamin D was dissolved in tween 80 and administered in dose of 12 I.U/kg i.p.

Experimental Design

We Selected 120 female mice having stress incontinence made the observation and noted the micturition timing. Among it for 80 breeding stage has passed and they are suffering from stress incontinence whereas 20 is taken as control group (10 each in two groups for both the category) and 20 is taken toxic control (10 each in two groups for both the category) for comparison purpose. They were further divided into two groups i.e., group I consisted of forty mice having mild SU stage I (i.e. incontinence when coughing, sneezing) and others in group II having moderate severe SU stage II (i.e. incontinence when coughing, sneezing, physical body movements, freely moving around, dropping urine at most hop).

Table 1: Stages of stress Incontinence

Stage I: low	urine loss quantity maximally until 10 g/24 hr.
Stage II: medium	urine loss quantity of 11 to 50 g/24 hr.
Stage III: massive	urine loss quantity over 50 g/24 hr.

Third degree of stress incontinence (excess urine loss) was prohibited from the study.

Dose Regimen

Subsequently we gave a combined intraperitoneal dose regimen of estriol (0.005 mg/kg) and vitamin D (12 I.U/kg) for 6 weeks. The mice were administered i.p thrice a week. Before and after 6 weeks serum level of vitamin D were determined by "mouse rat 25-oh vitamin D ELISA assay kit". Condition of the all the mice in both the groups were evaluated (protocol of micturition, PAD test).

Compliance found to be good and mice demonstrated to get on well with the study.

PAD Test

Before starting with our drugs, we performed PAD test which is described as standardized diaper test for 24 hours. The mice were taped with the piece of sanitary napkin and replaced every 12 hours (Uebbing et al., 2016). Scotch tape was brought to use so that no skin hair gets extracted from the mice body while removal of the pad. First, we measured the total amount of urine female mice passes and make an average of it. This value helped us to detect the weight of the urine passed due to stress incontinence.

Formula for calculation of amount of urine released due to stress incontinence

The used sanitary napkin were weighed, the unloaded weight and average weight was deducted and the urine quantities were computed from the below formula:

Weight of the urine passed due to stress incontinence was calculated from the below formula:

Weight of urine due to Stress Incontinence = Total weight of diaper - (Unloaded weight + average urine weight)

Statistical Analysis

Data are expressed as mean \pm standard error of mean (SEM). Statistical comparisons were made by using repeated measures analysis of variance (ANOVA) followed by Turkey's as post hoc multiple comparison test to observe differences among the groups. $P < 0.05$ were considered statistically significant.

Results and Discussion

The serum levels of vitamin D before therapy were measured in all female mice (n=50) degree of We made 6 groups of 10 mice selected in each category i.e SU1 and SU2 and in both the category control groups (normal control group and toxic control group) was introduced consisting of 10 mice. We have reported the results in format of before and after for comparison between both the categories.

PAD TEST

Here we observed the micturition parameter of all the mice and made an average and calculated the amount of urine passed due to stress incontinence. We are reporting the result in before and after case, i.e before the treatment begins and after the treatment gets finished.

Symptoms before the treatment in SU1:

In category SU1, animals were found to be suffering from stage I stress incontinence (i.e urine loss not more than 10g/hr). Before beginning of therapy we

found that group SU1a was leaking very minute amount of urine, group SU1b was moderate, group SU1c and SU1d were leaking on almost on extreme ends.

Symptoms after the treatment in SU1:

In category SU1, after the completion of treatment we found that group SU1a and SU1b recovered from the stress incontinence, while SU1c had marginally urine loss and SU1d has moderate improved result. Almost half of the animals : no more incontinence while coughing and sneezing. About one-fourth: less incontinence and problems, urine loss quantity was reduced while sneezing and coughing and rest little difference was noticed.

We conclude after end of the treatment, almost 75% of SU1 mice's condition improved and combination of estriol with vitamin D was of measurable benefit to them.

Symptoms before the treatment in SU2:

In category SU2, animals were found to be suffering from stage II stress incontinence (i.e urine loss not more than 11 to 50g/hr). Prior to beginning of therapy we found that group SU2a and SU2b pad test result were leaking elevated levels on almost body movement with sneezing and coughing too, whereas SU2c and Su2d leakage was very soaring high found on almost every hop and sneezing and coughing too.

Symptoms after the treatment in SU2:

In category SU2, after the completion of treatment we found that group SU2a and SU2b pad result reveals that urine level has drastically improved and is less than 11 g/hr which employs that its condition falls into category of stress incontinence level I (i.e urine loss not more than 10g/hr). We summarized our findings as follows;

Approximately half of the animals: stress incontinence has improved from stage II to I, among it about one-fourth has no more stress incontinence, while 25% depicted remarkable decreased urine loss problems and rest one-fourth exhibited no improvement (no difference in before and after of urine loss quantity in pad test)

After end of treatment we draw a conclusion that one-fourth from SU2 has no more incontinence and 37% of our animals our no more suffering from stress incontinence in both the groups. So far combination therapy of estriol and vitamin D is found to be beneficial to most animals in our study.

Serum levels of Vitamin D before the therapy in SU1 and SU2 category

SU1 category

We detected that animals were lacking in vitamin D. In category SU1 we discovered that only 10% of

animals had good amount of serum vitamin D and surprisingly 40% had unsatisfied level of vitamin D.

SU2 category

We estimated that animals were deficient in vitamin D. In category SU2 we came across that only 5% of animals had good amount of serum vitamin D and it was eye opener to know that 40% had unsatisfied level of vitamin D.

Serum levels of Vitamin D after the therapy in SU1 and SU2 category

SU1 category

Post treatment of 6 weeks with estriol and vitamin D we revealed that only 5% of animals has "unsatisfied" level of vitamin D which is in comparison with before treatment is 50% recovery. For "satisfied" group only 10% of animals fall into this which has decreased from 38% before the treatment, this data in itself represents the fruitful results of the therapy. For "sufficient" group now 35% of animals' falls into this which was earlier only 20%, it's a big margin recovery. For "good" group now 50% of animals comes under this, it's a 5-fold recovery from the before treatment data.

SU2 category

Post treatment of 6 weeks with estriol and vitamin D we revealed that only 21% of animals has "unsatisfied" level of vitamin D which is in comparison with before treatment approximately 50% recovery. For "satisfied" group 38% of animals falls into this which has increased from 35% before the treatment. For "sufficient" group now 31% of animals comes into this level of vitamin D. For "good" group we report that 10% of animals are under this bracket which has doubled from before the study. Most animals have recovered from unsatisfied level of vitamin D and now falls mainly into satisfied and sufficient group but some animals also have unchanged group of vitamin D level too.

Table 1. The standard values for 25-Hydroxy-Vitamin D in mice is given below:

Level of Vitamin D	Interpretation
<10ng/ml	Unsatisfying
10-20 ng/ml	Satisfying
20-30ng/ml	Sufficient
>30 ng/ml	Good

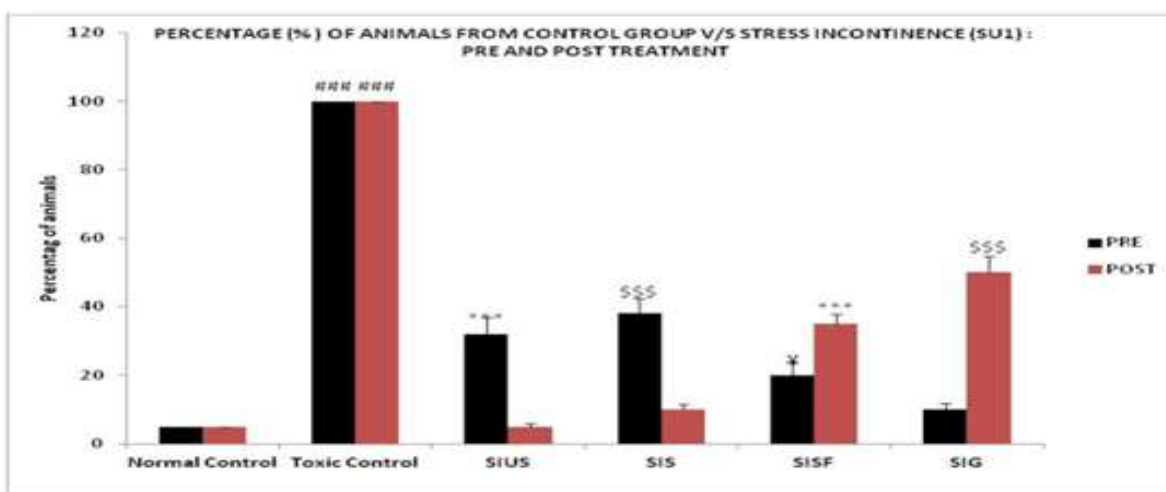
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Figure 1: Effects of combination of Estriol and Vitamin D on percentage of animals from control group v/s stress incontinence (SU1) pre and post treatments



Data are expressed as mean \pm standard error of mean (SEM). SIUS: Stress Incontinence Unsatisfying, SIS: Stress Incontinence Satisfying, SISF: Stress Incontinence Sufficient, SIG: Stress Incontinence Good. All values are expressed as mean \pm SEM (n=10 rats). ***P<0.001, ****P<0.001, *****P<0.001. ****P<0.001 when compared with normal control group, ***P<0.001 when compared with toxic control group. Statistical comparisons were made by using repeated measures analysis of variance (ANOVA) followed by Turkey's as post hoc multiple comparison test to observe differences among the groups. P<0.05 were considered statistically significant.

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