PREMENSTRUAL ESTRADIOL LEVELS IN FEMALE PATIENTS WITH ISCHEMIC STROKE AND POST-ISCHEMIC STROKE EPILEPSY

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ABSTRACT

Estrogen serves as preischemic treatment for cerebral ischemia protection and hence it is considered as vasoprotective and neuroprotective agent. The involvement of estrogen in epilepsy, ischemia, and other disorders has recently been comprehensively reviewed. Premenstrual estradiol 17-β serum levels (pmol/l) (Mean ± SEM) using ELISA kits in normal control (NC, n: 40) women subjects and women patients with ischemic stroke (IS, n: 37) and post-ischemic stroke epilepsy (PISE, n: 36) were found respectively as 221.125 ± 10.3989, 201.9459±8.6548 and 237.9722 ± 12.2337. Statistical analysis showed non-significant results for NC vs. IS and NC vs. PISE whereas IS vs. PISE showed significant variations (p=0.0183). One way ANOVA and Tukey-Kramer test for normal control subjects (NC) and subjects with ischemic stroke (IS) and post-ischemic stroke epilepsy (PISE) demonstrated significant variations among groups (Fs =2.856; p = 0.05). Tukey-Kramer test showed significant variations for ischemic stroke (IS) subjects vs. post-ischemic stroke epilepsy (PISE) subjects. Stress and depression, lack of physical activity and personal or family history of stroke or TIA presented significant difference ($p < 0.05$). Conclusively, more work is needed to be carried out to understand the precise role of premenstrual estradiol in ischemic stroke and post-ischemic stroke epilepsy.

Key-words: estradiol 17-β, premenstrual, risk factors, ischemic stroke, post-ischemic stroke epilepsy

INTRODUCTION

Estrogen serves as preischemic treatment for cerebral ischemia protection and hence it is considered as vasoprotective and neuroprotective agent. The involvement of estrogen in epilepsy, ischemia, and other disorders has recently been comprehensively reviewed (Pajarillo *et al*., 2019). Estradiol has been suggested as having effects on neuroprotection for stroke and related disorders, post-stroke epilepsy and other conditions (Sohrabji , 2015).

The occurrence of ischemic stroke was found affected by shift work schedules caused by biological sex (mainly serum estradiol levels) (Earnest *et al*., 2016). This has further been clarified that neuroprotective effects of preconditioning with estrogen in experimental MCAO (middle cerebral artery occlusion) occur (Liu and Pan, 2016). Decrease in ischemia-reperfusion injury was found to occur in response to endogenous estrogen (Zhang *et al*., 2017). It was revealed that estrogen therapy restored partially the vessel function to similar extent as nonischemic vessels (Goodrow *et al*., 2005). Acute estrogen therapy provides recovery in experimental stroke during early reperfusion (McCullough *et al*., 2001). Reduction in stroke injury occurs by 17 beta-estradiol in estrogen deficient female rats, though the precise mechanisms of such occurrence might not be identical (Rusa *et al*., 1999).

It has been investigated that estrogen provides protection against white matter injury and declarative memory deficits associated to chronic cerebral hypoperfusion (Dominguez *et al*., 2018). Indirect experimental investigation shows that oophorectomy decreases estrogen levels and neurovascular recovery in female rat focal ischemic stroke (Bazzigaluppi *et al*., 2018). Another report shows that estrogen deficiency may lead to the development of stroke in postmenopausal women (Yeasmin *et al*., 2017).

However, reports showing results contrary to the mentioned investigations are also documented. Serum estradiol was found not changed after cerebral ischemia (Zhao *et al*., 2012). The circulating estrogen concentration

was decreased but it did not influence the cerebral vasodilatory capacity as stroke associated property (Bain *et al*., 2005). Estrogen has been indicated possibly as a major factor in female resistance to ischemia (Liao *et al*., 2001). It is known that common carotid arteries and right middle cerebral artery are occluded followed by reperfusion, but post-ischemic alterations occur inversely correlating with circulating estrogen concentrations, and decline in estrogen levels occurs in view of post-ischemic changes (Liao *et al*., 2001).

It has been suggested that high concentration of estradiol is a new predictor in IAD (ischemic artery disease) in older postmenopausal women (Scarabin-Carré *et al*., 2012). Estrogen is released in parabrachial nucleus (PBN) of male rat after middle cerebral artery occlusion (MCAO) (Saleh *et al*., 2004). It has been suggested that middle cerebral artery (MCA) occlusion causes the synthesis of estrogen for preventing MCA induced autonomic dysfunctions (Saleh *et al*., 2005).

Inconsistent results have also been found for the effects of beta-estradiol- both increasing and decreasing the sensitivity to ischemia/ cerebral ischemia (Harukuni *et al*., 2001).

Estrogen (17β-estradiol or E2) regulates various mechanisms in cerebral ischemia by controlling/ influencing post-ischemic alterations and diseases including epilepsy (Scott and Brann, 2013). Cerebral ischemia, epilepsy and a variety of other disorders are influenced by estrogen and other sex steroids (Vagnerova *et al*., 2008).

The literature documents the decrease or increase in estradiol concentrations in ischemic stroke and post-stroke alterations, but there are reports not finding any change in estradiol levels after cerebral ischemia (Zhao *et al*., 2012) or decreased estrogen levels in ischemic stroke not influencing the cerebral vasodilatory capacity as stroke associated property (Bain *et al*., 2005).

Our previous studies related to estrogen, premenstruation, ischemic stroke and epilepsy (Hussain *et al*., 1987, 1993a,b, 1995a, b, 2006, 2007; Qureshi *et al*., 1988; Hussain, 1991, 2010; Naz *et al*., 2009; Hussain and Zahir, 2012) provide important information for carrying out further studies.

In view of above mentioned contradictory studies, we planned to carry out present study to investigate the alterations in premenstrual estrogen levels in women with ischemic stroke and women with post-ischemic stroke epilepsy, though gender-based information requires further studies to be done.

MATERIALS AND METHODS

Normal control (NC) women subjects (n: 40), ischemic stroke (IS) women subjects (n: 37), and post-ischemic stroke epilepsy (PISE) women subjects (36) were selected for the present study. The average age of the subjects was 55 years.

Some of the records taken for diagnostic purpose in patients with ischemic stroke, and post-ischemic stroke epilepsy as well as normal controls as applicable were: Age, personal habits, smoking habits, history of patients, previous and current medication, physical and general examination, major risk factors, measurement of body weight and height and calculation of body mass index (BMI), blood pressure (systolic/ diastolic), pulse & temperature, total protein, total cholesterol, LDL-Cholesterol, HDL cholesterol, menstrual cycle records, and other relevant physiological and biochemical profiles. Inclusion and exclusion criteria were set. The patients with ischemic stroke and post-ischemic stroke epilepsy were selected after thorough clinical history assessments. They were categorized according to their age and the risk factors they had.

The risk factors (smoking, obesity, hypertension, diabetes, heart disease, stress and depression, lack of physical activity and personal or family history of stroke or TIA etc) were determined in number and percent data for the subjects. The t-test was analyzed by Z-scores for percentage data.

For the determination of premenstrual serum estradiol (using ELISA kits), the pilot studies were carried out initially (not included in this manuscript) in ischemic stroke and post-ischemic stroke epilepsy. Only 1-2 days prior to the start of menstruation was considered as premenstrual part for the present study. Once the methods were established with inter and intra assay variations, new subjects were selected for thorough studies.

For the statistical analysis, spreadsheets were used instead of statistical analysis in SAS or SPSS. The p value was considered as significant at $p < 0.05$. Premenstrual estradiol 17-β serum levels (pmol/L) (Mean ± .SEM) were determined in normal control (NC, n: 40) women subjects and women patients with ischemic stroke (IS, n: 37) and post-ischemic stroke epilepsy (PISE, n: 36). The t and p values were found and comparisons for NC vs. IS, NC vs. PISE and IS vs. PISE were carried out. One way ANOVA and Tukey-Kramer test were employed for comparing the data of normal control subjects (NC) and subjects with ischemic stroke (IS) and post-ischemic stroke epilepsy (PISE).

RESULTS

Premenstrual estradiol 17-β serum levels (pmol/l) (Mean±SEM) in normal control (NC, n:40) women subjects and women patients with ischemic stroke (IS, n:37) and post-ischemic stroke epilepsy (PISE, n:36) were respectively as 221.125± 10.3989, 201.9459±8.6548 and 237.9722± 12.2337 (Table 1).

Table 1. Premenstrual estradiol 17-β serum levels in women with ischemic stroke and post-ischemic stroke epilepsy.

	Normal control (NC) subjects	Ischemic stroke (IS) subjects	Post-ischemic stroke epilepsy (PISE) subjects
Total subjects	40	37	36
Mean Age (yrs)	55.5	56	54.5
E2 levels (pmol/L) (Mean ± SEM)	221.125 ± 10.3989	201.9459 ± 8.6548	237.9722 ± 12.2337

E2: estradiol 17-β; NC: normal control subjects; IS: ischemic stroke subjects; PISE: post-ischemic stroke epilepsy subjects

Statistical analysis showed non-significant results for NC vs IS and NC vs PISE whereas IS vs PISE showed significant variations (p=0.0183) (Table 2).

Table 2. Significance difference for premenstrual estradiol 17-β serum levels among normal control women and women with ischemic stroke and post-ischemic stroke epilepsy.

	NC vs. IS	NC vs. PISE	IS vs. PISE
t-value	1.4055	1.0556	2.4153
p-value	0.1640	0.2946	0.0183

NC: normal control subjects; IS: ischemic stroke subjects; PISE: post-ischemic stroke epilepsy subjects

One way ANOVA and Tukey-Kramer test for normal control subjects (NC) and subjects with ischemic stroke (IS) and post-ischemic stroke epilepsy (PISE) demonstrated significant variations among groups (Fs=2.856; p=0.05; Table 3). Tukey-Kramer test showed significant variations for ischemic stroke (IS) subjects vs post-ischemic stroke epilepsy (PISE) subjects (Table 3).

Table 3. One way ANOVA and Tukey-Kramer test for normal control subjects (NC) and subjects with Ischemic stroke (IS) and Post-ischemic stroke epilepsy (PISE).

	Sum of squares	Degrees of freedom	Mean square	Fs	p	Variance component (%)
Among groups	23733.664	2	11866.832	2.856	0.05	4.70
Within groups	457041.239	110	4154.92			95.30
Total	480774.903	112				
	NC	IS	PISE			
Mean	221.125	201.945946	237.972222	-	-	-
n	40	37	36	-	-	-
Tukey-Kramer test						
Normal subjects-Ischemic stroke subjects					not sig	
Normal subjects-Post-ischemic stroke epilepsy subjects					not sig	
Ischemic stroke subjects-Post-ischemic stroke epilepsy subjects					sig	

Main risk factors for patients with ischemic stroke (IS) and post-ischemic stroke epilepsy (PISE) are given in Table 4.

Table 4. Main risk factors for patients with ischemic stroke (IS) and post-ischemic stroke epilepsy (PISE).

Main Risk factors	Ischemic stroke (IS) subjects (n: 37)		Post-ischemic stroke epilepsy (PISE) subjects (n: 36)		Significance	
					Z-value	p-value
	n	%	n	%		
Smoking	21	56.76	13	36.11	1.768	0.077
Obesity	20	54.05	16	44.44	0.821	0.413
Hypertension	17	45.95	12	33.33	1.101	0.272
Diabetes	12	32.43	7	19.44	1.2644	0.208
Heart disease	19	51.35	14	38.89	1.0696	0.285
Stress and depression	17	45.95	5	13.89	2.9843	0.003
Lack of physical activity	7	18.92	20	55.56	-3.2417	0.001
Personal or family history of stroke or TIA	17	45.95	4	11.11	3.2871	0.001

Stress and depression, lack of physical activity and personal or family history of stroke or TIA presented significant difference ($p<0.05$; Table 4). All other risk factors recorded did not show statistically significant results in the present study.

DISCUSSION

The present study provides evidence that cerebral ischemia may cause reduction in premenstrual serum estradiol levels whereas post-stroke epilepsy elevates the serum levels of estradiol. This information has previously been documented where estrogen was used as preischemic treatment for ischemic stroke and/ or post-stroke epilepsy (Rusa *et al*., 1999; McCullough *et al*., 2001; Goodrow *et al*., 2005; Vagnerova *et al*., 2008; Sohrabji , 2015; Liu and Pan, 2016; Dominguez *et al*., 2018), oophorectomy (Bazzigaluppi *et al*., 2018) or endogenously (Zhang *et al*., 2017), postmenopausaly (Scarabin-Carré *et al*., 2012), post-ischemic alterations (Liao *et al*., 2001) or estrogen synthesis in response to MCA occlusion (Saleh *et al*., 2005).

Most of the studies mentioned in above paragraph provided interesting but experimental aspects of the role of estrogen in ischemic stroke and / or post-stroke epilepsy. However, the present study is a well controlled and completely clinical investigation. It gives emphasis for investigating further about how the levels of premenstrual estradiol are declined (though non-significantly) in women patients with ischemic stroke compared to those in normal healthy control women, and significantly higher in post-stroke epilepsy patients compared to ischemic stroke patients.

More important investigation in the present study relates to significant elevation of premenstrual serum estradiol in post-stroke epilepsy patients as compared to estradiol levels in women with ischemic stroke. These observations can be explained and interpreted with the help of relevant studies carried out previously (Harukuni *et al*., 2001; Liao *et al*., 2001; Vagnerova *et al*., 2008; Scott and Brann, 2013; Bazzigaluppi *et al*., 2018), although more work is needed to be carried out to understand the precise role of estradiol in ischemic stroke and post-ischemic stroke epilepsy.

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