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Original Research Article

A HOSPITAL BASED PROSPECTIVE STUDY TO EVALUATE THE ASSOCIATION OF PLACENTAL TISSUE ESTROGEN RECEPTOR ALPHA GENE EXPRESSION WITH POSTPARTUM DEPRESSION IN WOMEN AT RISK AT TERTIARY CARE CENTER

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Abstract

Background: Women are at higher risk of developing depression than men. There is no single cause of PPD in women, but physical and emotional issues play a major role. Accumulative evidence suggests the role of estrogen in depression. Serotonin and nor adrenaline are thought to play key roles in depression. The purpose of our study is to determine the expression of placental ESR α in women with postpartum depression versus normal postpartum women in order to establish an association between placental ESR α and postpartum depression so that it can be taken as a predictor for PPD. **Materials and Methods:** A hospital based prospective study done in all pregnant women of any age, parity and who were willing to participate in the study till the completion were enrolled for the study in third trimester if they had at least one of the risk factors according to Antenatal Postpartum Depression Predictors Inventory-Revised (PDPI-R)12during one year period. All enrolled women were followed at 2 weeks and 6weeks after delivery for any symptom of postpartum depression. All women were assessed by Edinburgh Postnatal Depression Scale (EPDS) Hindi version for postpartum depression.13 At the end of the study, stored placental tissue of both cases and controls, was analyzed for ESR α gene expression and the quantitative levels of ESR α RNA expression. **Results:** Women with score ≥10 were considered for psychiatric evaluation for confirmation of depression and then they were selected as cases. After matching equivalent number of women with EPDS score <10 was selected as controls. Gene expression analysis was done for both cases and controls, on stored placental tissue. Women developing PPD after delivery are having significantly lower expression of ESR α gene in their placenta at the time of delivery which is 1.33 times less as compared to women who remain healthy. Mean ESR α delta ct value in PPD cases (3.33±0.697SD), was significantly higher than the controls (2.91±0.759SD), with p value 0.032. **Conclusion:** We can conclude that down regulation of placental ESR α gene expression at delivery plays a crucial role in PPD. Further studies are needed to disclose placental ESR alpha signaling can be utilized in devising ESR α receptor agonists to prevent PPD.

INTRODUCTION

Postpartum depression according to International Classification of Disease 10 (ICD 10) is defined as the mental and behavioral disorder commencing within 6 weeks of delivery, that do not meet the criteria for disorder classified elsewhere.[1] Many women experience self-limited and mild symptoms, but postpartum depression should be suspected when symptoms are severe and have lasted over two weeks. It usually begins between two weeks to a month after delivery.[2] Recent studies have shown that 50% of postpartum depressive episodes actually begin prior to delivery.[3] Therefore in the Diagnostic & Statistical Manual of Mental Disorders 5th Edition (DSM V), postpartum depression is diagnosed under "depressive disorder with peripartum onset", in which peripartum onset is defined as anytime either

during pregnancy or within the four weeks following delivery. PPD may last several months or even a year after delivery.[4]

The overall prevalence of PPD has been reported up to 60% depending on the population and study method.[5] Prevalence of PPD in Indian population was found to be 15.8% attending postnatal clinic.[6]

Women are at higher risk of developing depression than men. After adolescence incidence of depression in women is twice as compared to men. While the relative risk of depression changes throughout the women's reproductive lifecycle, the windows of increased vulnerability occur during periods of significant hormonal fluctuation.[7] Postpartum period is particularly important as depression at that time not only affects the women but mother - child bonding, husband -wife relationship, interpersonal relations and whole family also gets affected. Depressed mothers may experience unnecessary suffering, deteriorating health status, marital discord, and suicidal ideation. Offspring of these depressed mothers have been shown to have significant emotional, cognitive, social, behavioral and developmental delays, as well as an increased risk for mental disorders, which can persist throughout childhood. By considering all this PPD has been called "the most significant obstetrical complication after delivery" and the illness was recently elevated to a "global health challenge" by the WHO.[8]

PPD is a neglected condition in a developing country like India as it does not produce any gross symptoms and hence it is detected late or even may not be detected. Delay in delivering adequate treatment for PPD is unfortunate as the response to treatment is good, so effective detection becomes an important thing which requires a good coordination of a wide variety of primary and secondary services including midwives, health visitors, clinical psychologist and obstetrician.

As preventive measures, early intervention and identification can alleviate suffering of the mother and minimize its potential harmful effects on the newborn. So, the first step in preventing PPD is the identification of women who are at risk for developing it. There is no single cause of PPD in women, but physical and emotional issues play a major role. Physical changes occurring after childbirth due to a dramatic drop in hormones (estrogen, progesterone, thyroid, serotonin levels) can contribute to PPD. Emotional factors include sleep deprivation, anxiety about newborn care and her physical appearance, struggle with sense of identity, or feel that she has lost control over her life any of these issues can contribute to PPD.[9]

Accumulative evidence suggest the role of estrogen in depression. Serotonin and nor adrenaline are thought to play key roles in depression. Estrogen regulates serotonin receptor number and function9 controlling the activity of serotonergic neurons. Acute drop in the level of estrogen following expulsion of estrogen secreting placenta thus may cause sharp fall in serotonin levels leading to postpartum depression.

Estrogen exerts its biological effects in large part through intracellular activation of its principle receptors estrogen receptor alpha (ESR α) and estrogen receptor beta (ESR β), but ESR α plays a more important role than ESR β in emotions and mood.[10]

Thus, expression of ESR α gene can affect the whole pathway and lead to postpartum depression. ESR α has been documented in brain, reproductive organs (ovary, uterus & cervix) and cytotrophoblast cells (CT) of the placenta.[11]

Many women sufferings from postpartum depression fail to seek help due to social stigma and inadequate social support in a developing country like India. The purpose of our study is to determine the expression of placental ESR α in women with postpartum depression versus normal postpartum women in order to establish an association between placental ESR α and postpartum depression so that it can be taken as a predictor for PPD.

MATERIALS AND METHODS

A hospital based prospective study done in Department of Obstetrics and Gynecology in collaboration with Molecular Biology Laboratory, Department of Biochemistry and Department of Psychiatry at University College of Medical Science (UCMS) & Guru Teg Bahadur Hospital (GTBH), Delhi during one year period.

Inclusion Criteria

All pregnant women of any age, parity and who were willing to participate in the study till the completion were enrolled for the study in third trimester if they had at least one of the following risk factors according to Antenatal Postpartum Depression Predictors Inventory-Revised (PDPI-R):[12]

- Single parent: divorced, widow, separated
- Low socio-economic status (According to Modified Kuppuswamy Scale)
- Low self-esteem or negative self-estimation (does not feel good/worthwhile/no good qualities)
- Past history of documented depression
- History of prenatal anxiety (feeling of uneasiness or apprehension concerning a vague nonspecific threat)
- Unplanned or unwanted pregnancy
- Lack of social support (parents working, nuclear family, poor socio emotional support from paternal or maternal side, dependent on maid etc.)
- Marital dissatisfaction (affection, communication, mutual activities)
- History of life stress

Exclusion Criteria

- Women with diagnosis of depression in current pregnancy
- Women with a history of psychiatric illness other than depression

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Selection of Control

- For each case, age, religion and residence were matched. Pregnant women in their third trimester matched with cases were recruited as controls.

Methodology

Ethical clearance was obtained from the institutional ethical clearance committee for human research. An informed written consent was taken from all women after enrolment. In third trimester eligible antenatal women were enrolled for the study and a detailed history with emphasis on socio demographic status, obstetric history including planned or unplanned pregnancy, marital relationship and previous history of depression was taken.

Immediately after delivery of placenta, a piece of 1×1cm. size of placenta was dissected out from maternal surface of the placenta, from periphery. Sample was collected in a sterile culture vial containing 5 ml phosphate buffered saline (PBS) and initially stored in the freezer compartment of the labor ward fridge before transporting to biochemistry. The samples were transferred to biochemistry department within 24 hrs of collecting it, where they were stored in freezer at -800c for later estimation of ESRα gene expression.

All enrolled women were followed at 2 weeks and 6weeks after delivery for any symptom of postpartum depression. History of postpartum maternal morbidity or neonatal morbidity and mortality if any was documented in Performa at the time of visit. All women were assessed by Edinburgh Postnatal Depression Scale (EPDS) Hindi version for postpartum depression.13 EPDS is a 10-item, screening questionnaire for postpartum depression. It records the mental status of the mother, and how she felt during the last seven days. Each item has four possible responses, which are scored from 0-3 depending on the severity of the responses. Higher scores indicate more severe depressive symptoms with a maximum total score of 30. The recommended cut-off point is 9/10. A score of 10 or higher may indicate that depression symptoms have been reported.

Scoring

- Q 1, 2 and 4- are scored 0, 1, 2 or 3 with top box scored as 0 and bottom box scored as 3.
- Q 3, 5-10- are reversed score with top box scored as 3 and the bottom box scored as 0.

Maximum score: 30

Possible depression: 10 or greater

Always look at item '10' (suicidal thoughts)

At 2 week follow up, women were screened for PPD by this EPDS scale. Women scoring 10 or greater were taken to psychiatrist for evaluation and to confirm the postpartum depression as per ICD-10 criteria. Women who were confirmed with postpartum depression were selected as cases. Women with EPDS score less than 10 were followed up at 6 weeks and again screened at 6 weeks for PPD by EPDS. Women scoring 10 or greater were taken to psychiatrist for confirmation. After confirming they were taken as cases. Equivalent number of women scoring less than 10 was selected as controls. At the end of the study, stored placental tissue of both cases and controls, was analyzed for ESR α gene expression and the quantitative levels of ESR α RNA expression evaluated by the following steps:

i) RNA Extraction: RNA extraction was done using Trizol reagent. The tissue sample (1×1 cm) was homogenized (using an automated homogenizer machine) in 1ml Trizol reagent at room temperature and cold centrifuged at 12,000×g for 10 minutes. The clear supernatant was transferred to a fresh tube and 200 ml of chloroform was added followed by vigorous shaking of the tube for 10-15 seconds. Then the samples were centrifuged at 12,000× g for 15 minutes at 40C. The Upper aqueous phase was collected into a fresh micro centrifuge tube and 0.5 ml of isopropanol was added, the tube was incubated at room temperature for 10 min, followed by cold centrifuge at 12,000×g for 10 minutes. The supernatant was discarded, and the pellet was washed with 1 ml 75% ethanol and centrifuged at 7500×g for 5 minutes at 40C. The pellet was then air dried for 5-10 minutes at room temperature and was resuspended in 30mL of RNase free water and stored at -800C until further use. The quantity of RNA was measured on nano drop spectrophotometer (ND2000, Thermo scientific USA).

ii) cDNA Synthesis: From the total RNA extracted mRNA was used as a template for the synthesis of complementary DNA (cDNA) using Verso cDNA synthesis kit (Thermo scientific, USA). The synthesized cDNA was stored at -80°C. This cDNA was used to study ESR expression by Real Time quantitative PCR (RT-qPCR).

iii) Quantitative expression of ESR alpha gene by real time PCR:

Real time PCR (Biorad Connect) was used to measure the expression of ESR alpha gene in placental tissue of PPD cases and controls by determining delta Ct(cycle threshold).

Primer sequences used for ESR α are as followed:

Genes	Forward	Reverse
ESR α	5'-AGCCAGTGCAATCAACACGA-3'	5'-GGTGCTCACTGGATTACCTGAA-3'
Gapdh	5'-CCAAGGTCATCCATGACAACTTTGGT-3'	5'-TGTTGAAGTCAGAGGAGACCACCTG- 3'

Reaction setup: The reaction mixture of 20µl of a sample for a gene was made as follows:

Reagents	Volume (µl)
Master Mix	10
Primer Forward	1
Primer Reverse	1
cDNA	1
Nuclease Free Water (NFW)	7

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Following PCR reaction condition were followed for the amplification of ESR alpha gene:

Gene	Initial Denaturation	Denaturation	Annealing	Melt Curve
ESR α	950C, 5 min	950C, 30 sec (39 cycles)	600C, 30 sec (30 cycles)	60-95°C

In the initial cycles of PCR, there is a little change in fluorescence signal (produced from double stranded DNA). This defines the baseline for the amplification plot. An increase in fluorescence above the baseline indicates the detection of accumulated target. The parameter Ct (cycle threshold) is defined as the fractional cycle number at which the fluorescence passes the fixed threshold. Ct levels are inversely proportional to the amount of target nucleic acid in the sample i.e. lower the value of Ct, higher the amount of target nucleic acid in the sample. In this study, expression normalization was done using Gapdh gene to correct sample to sample variations in RT-PCR efficiency and errors in sample quantification. ΔCt was evaluated which is the difference of average Ct of target gene (ESR α gene) in case and their constitutive gene (Gapdh gene):

ΔCt = Average Ct target - Average Ct reference.

Again, the difference of mean Ct values of control and cases was determined, which is **delta-delta Ct (ΔΔCt).** **ΔΔCt =ΔCt (case) - ΔCt (control)**

After this, true Fold change (FC) was represented to compare the expression of genes between cases and controls by the following formula:

FC= 2 $^{-\Delta\Delta Ct}$.

Statistical Analysis: Microsoft Excel (version 2007) and statistical software SPSS for windows (version 20.0) was used for data presentation and statistical analysis. P-value<0.05 was considered as significant.

RESULTS

Our study showed that the mean age of subjects in cases was 25.57 (±2.77 SD) years and in controls was 26.23(±3.35 SD) years. Majority of women were Hindu by religion followed by Muslim. Both the groups were comparable with respect to age & religion was statistical non-significant (P=1.000 & P=0.22) [Table 1].

Socio economic status scoring was done by revised Kuppuswamy Classification based on education, occupation and monthly income of family. Maximum number of the women coming to GTB Hospital belonged to the middle class followed by lower class. Among PPD women majority (53.4%) belonged to lower class whereas among controls majority (76.7%) belonged to middle class. A statistically significant association was found between postpartum depression and lower socio-economic status. PPD was more likely in women with lower socio-economic status (p value <0.001) [Table 1].

Majority of women belonged to joint family in both case (18%) and control group (27%). Among PPD cases 60% belonged to joint families & 40 % belonged to nuclear families. Among controls 90% belonged to joint families and only 10% to nuclear families. A statistically significant association (p value< 0.001) was found between nuclear family and postpartum depression. This finding suggests that the joint family may add as a protective factor for PPD [Table 1].

Majority of women participated were married in both case (93.4%) and controls (100%) groups. Only 2 participants were separated, and none were single, widowed, partnered or divorced [Table 1].

Majority of women had duration of marriage within 10 years in both PPD cases and controls. No statistical significance (p value 0.064) was found between duration of marriage and PPD [Table 1].

Analysis of social factors contributing to postpartum depression [Table 2]:

Gender preference: Majority of women in both PPD case and control groups had preference for male child but no significant association (p value 0.071) was found between PPD and gender preference.

Pressure for male child: Among PPD cases out of 30, 63.4% had family pressure for male child and among control group only 10% had such pressure. Significant association (p value <0.001) was found between PPD and family pressure for male child. Depression was more likely in study participants having any pressure for male gender.

Independent decision-making power: Among PPD cases, only 20% and among control group 80% had some decision-making authority. Significant association (p value <0.001) was found between PPD and decision-making power. Depression was more likely in study participants having no decision-making power. None of the women in the case and control groups had the independent decision-making authority.

Self-esteem: 56.7% women in PPD group and 96.7% in control group had positive self-esteem. Significant association (p value <0.001) was found between PPD and low self-esteem of the study groups. Postpartum Depression was more likely in study participants having low self-esteem.

Partner support: Among PPD cases 40% women had no support from their spouse and among control group all women were receiving adequate support from their partner. A significant association (p value <0.001) was found between PPD and partner's support. Depression was more likely in study participants who had no support from their partner.

Family support: Among PPD cases, only 36.6% and among control groups, 86.6% had good family support. Significant association (p value <0.001) was found between PPD and support from the family. Depression was more likely in women having no family support.

Life stress: Among PPD cases, 53.3% women had some life stress factor as compared to only 10 % women in control group. Life stress factors included financial problems, marital problems, any illness or death in the family, and unemployment. Significant

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association (p value <0.001) was found between PPD and stress factors in life.

Domestic violence Majority of women in both PPD case and control groups had no history of domestic violence. Among PPD cases, 26.6 % and control group 6.6% had history of domestic violence. Significant association (p value <0.038) was found between PPD and domestic violence. Depression was more likely in study participants having history of domestic violence with them.

Our study showed that ESRα gene was found to be significantly down regulated by 1.33 times among cases as compared to controls. Mean ESR α delta ct value in PPD cases (3.33±0.697SD), was significantly lower than the controls (2.91±0.759SD), with p value 0.032. Among risk factors, only antenatal anxiety (negative correlation, correlation coefficient -0.548 and p value 0.002) and marital satisfaction (positive correlation, correlation coefficient 0.462 and p value 0.010) had a significant association with ESR α gene expression [Table 3].

Table 1: Socio demographic profile of cases and controls (n=60).

	n=60	Case (30)	Control (30)	P value
Age distribution (years)				
20-25	28	14(46.7%)	14(46.7%)	1.000*
26-30	30	15(50%)	15(50%)	
31-35	1	1(3.3%)	0(0%)	
>35	1	0(0%)	1(3.3%)	
Religion				
Hindu	44	20(66.7%)	24(80%)	0.222
Muslim	14	9(30%)	5(16.7%)	
Sikh	2	1(3.3%)	1(3.3%)	
Christian	0	0(0%)	0(0%)	
Socio economic status				
Upper	0	0(0%)	0(0%)	<0.001
Upper middle	8	4(13.3%)	4(13.3%)	
Lower middle	30	7(23.3%)	23(76.7%)	
Upper lower	19	16(53.4%)	3(10%)	
Lower	3	3(10%)	0(0%)	
Type of family				
Joint	45	18(60%)	27(90%)	<0.001*
Nuclear	15	12(40%)	3(10%)	
Marital status				
Married	58	28(93.4%)	30(100%)	0.492*
Separated	2	2(6.6%)	0(0%)	
Single/Widow/Partnered/Divorced	0	0(0%)	0(0%)	
Duration of marriage(years)				
0-5	28	13(43.4%)	15(50%)	0.064*
6-10	23	9(30%)	14(46.7%)	
11-15	8	7(23.3%)	1(3.3%)	
>15	1	1(3.3%)	0(0%)	

*Fisher's exact test

Table 2: Social factors contributing postpartum depression

	n=60	Case (30)	Control (30)	P value
Gender preference				
Male	43	24(80%)	19(63.4%)	0.071*
Female	13	3(10%)	10(33.3%)	
No preference	4	3(10%)	1(3.3%)	
Pressure for male child				
Yes	22	19(63.4%)	3(10%)	<0.001
No	38	11(36.6%)	27(90%)	
Independent decision-making power				
Yes	0	0	0	<0.001
No	30	24(80%)	6(20%)	
Together with the husband	30	6(20%)	24(80%)	
Self esteem				
Low	14	13(43.3%)	1(3.3%)	<0.001
Positive	46	17(56.7%)	29(96.7%)	
Partner support				
Yes	48	18(60%)	30(100%)	<0.001
No	12	12(40%)	0(0%)	
Family support				
Yes	37	11(36.6%)	26(86.6%)	<0.001
No	23	19(63.4%)	4(13.4%)	
Life stress				
Yes	19	16(53.3%)	3(10%)	<0.001
No	41	14(46.7%)	27(90%)	
Domestic violence				

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Yes	10	8(26.6%)	2(6.6%)	0.038
No	50	22(73.3%)	28(93.3%)	

*Fisher's exact test

Table 3: Correlation of placental estrogen receptor alpha gene expression with each risk group of Annexure III in PPD women

	Spearman's rho correlation coefficient	P value
Socio economic status	-0.027	0.889
Marital status	0.046	0.808
Self esteem	-0.280	0.134
Antenatal anxiety	-0.548	0.002
Planned pregnancy	-0.141	0.457
History of depression	0.290	0.120
Partner support	0.177	0.350
Family support	0.284	0.129
Marital satisfaction	0.462	0.010
Life stress	-0.207	0.272

DISCUSSION

The study was started with antenatal women in their third trimester and women were enrolled according to the selection criteria and recruitment policy. To achieve 30 cases 209 women were screened. Out of which 16 were lost to follow up and 3 patients excluded because of controversy, finally 190 patients were followed at 2 weeks and 6 weeks to achieve 30 cases of Postpartum Depression. There are very few studies on postpartum depression and among them; studies for gene expression and postpartum depression correlation are very much less. This study provides information on the proportion of postpartum depression; various risk factors associated with postpartum depression & placental estrogen receptor α gene expression, and the association of placental estrogen receptor α gene expression with postpartum depression.

The worldwide prevalence of PPD ranges from 1% to 73.7% using various tools and rating scales.[14,15] The variability of prevalence of PPD can be explained by the fact that different studies had employed different scales and at different time in the postpartum period. It may also be influenced by the cross-cultural differences in different regions, differences in perception of mental health and stigma associated with it.

The proportion of postpartum depression in our study was found to be 8.9% at 2 weeks and 16% at 6 weeks as measured by EPDS taking score 9 as a cut off for PPD. The prevalence of postpartum depression has been reported to range from 3.2% to 48.5% in different studies conducted in India.[16,17]

The low prevalence of postpartum depression found by Gokhale et al may be due to the fact that they had used a high cut-off of 12 for EPDS.[16] They screened around 200 patients on 1st postnatal day and followed them on 6th postnatal day and 6th postnatal week. Taking 12 as the cut off for PPD prevalence of PPD was found to 11%on 1st day postpartum, 7.4% at 6th day postpartum & 3.2%at 6 weeks postpartum.

The prevalence of postpartum depression in Delhi was 15.8% (out of 202 women 32 were diagnosed with depression) as reported by Gupta et al,[18] which is almost near to the findings of our study even though both the studies differed in the scale used as screening tool. Gupta et al had conducted the study in a tertiary hospital of Delhi on women who had attended postnatal clinic at 6 weeks postpartum using Prime MD Today whereas in our study we used EPDS as the screening tool.[19]

Our study showed that the mean age of subjects in cases was 25.57 (±2.77 SD) years and in controls was 26.23(±3.35 SD) years. No statistical significance was found between age and postpartum depression (p value 1.000). Age was not found to be a risk factor for the development of postpartum depression in our study. This is similar to the results of the study by some other researchers who observed no association between age and postpartum depression.[17]

When a new baby is born there is a decrease in disposable income due to the added financial responsibilities that come along with the addition of a new member. This means less finance to spend on their needs and leisure activities. We found a significant association (P value <0.001) of postpartum depression and poverty status of study participants. Majority of the cases developing PPD (53.4%) belonged to the lower class whereas among controls majority (76.7%) belonged to middle class. Comparable findings have been reported by other researchers also. Similarly, Silva et al,[20] found a positive association between lower socioeconomic status and postpartum depression. He studied a cohort of 1109 women in their prenatal and postnatal periods. He concluded that lower socio-economic status was significantly associated with PPD (p=0.020, RR 1.76, 95%CI).

A statistically significant association (p value< 0.001) was found between nuclear family and postpartum depression. So, we can conclude that living in a joint family was a protective factor for PPD. Mothers in joint families get more support during physically and mentally stressful postpartum period. Mothers in nuclear families have to fend for themselves for their needs or depend on their husbands to do household chores unless some relatives or family members come over for their help. Similar findings of protective effect of joint family

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have been reported by other researchers in Pakistan.[21] Rahman et al,[22] demonstrated that in Pakistan, there is a positive influence of the traditional extended family and associated cultural practices like 'chilla'. In this practice, post-partum women are confined for 40 days, and all the household chores are performed by other female members of the family. Rahman et al,[22] screened the antenatal women in their third trimester of pregnancy at 6 weeks before delivery and at 10- 12 weeks after delivery. Point prevalence of ICD 10 depressive disorder was 25% in antenatal and 28% in post-natal period. Depressed women had poorer social and family support than non-depressed mothers. Also support from the child's grandmother was observed to be a protective factor in the development of postpartum depression.

Majority of women participated were married in both case (93.4%) and controls (100%) groups. Only 2 participants were separated, and nobody was single, widowed, partnered or divorced. In our study marital status was not found to be significant. The reason for this may be because of less number of single mother participated in our study. Contrary to this Ghosh et al,[23] found significant association between single mother and postpartum depression (widow 96% unmarried 100%, separated 100% showing higher preponderance).

Duration of marriage was observed to be a non-significant factor for the occurrence of postpartum depression. Similar results were observed in the study by Paykel et al and Mahmud et al in which no association was found between postpartum depression and duration of marriage.[24,25]

As reported in previous studies, the number of pregnancies can have a profound effect on mental health. It can reflect a higher care burden and psychological stress. Although, we observed that first time mothers were less likely to be depressed than those who had their second or third child similar to other studies, but it was not found statistically significant may be because of small sample size. There are some studies in which significant association was found between PPD and gravidity.[26-28] It is likely that the first child is not treated as a burden, and they are welcomed with more joy than second or third child. However, if the child is of the next birth order he/she may be considered an added burden and responsibility which may lead to more stress and possible depression. However some researchers have found no association of the number of pregnancies with postpartum depression.[24]

We have not observed any significant association between postpartum depression and disturbed sleep. This has been supported by Paykel et al,[24] who have also not found any relationship between postpartum depression and loss of sleep due to the child. Difficulty in sleep is not predictive of postpartum depression in women who have no history of previous depression.[29]

Women have more postoperative physical problems after a caesarean section than a normal delivery. In one study it has been stated that women with normal vaginal delivery have a better quality of life than those women who have a caesarean delivery.[30] Some researchers have found a significant increase in the prevalence of depression in women who underwent caesarean section. Contrary to this, we did not observe any association between postpartum depression and mode of delivery. Similarly no association was found by Mahmud et al.[25] Another study done by Mazaheri et al,[31] they found that the prevalence of postpartum depression to be more in women who had a normal vaginal delivery.

Indian society is a male dominated society. Males are accorded higher status than women and gender inequality is deeply prevalent. Due to this there is constant pressure on the women to have a male child. It may lead her to worry more about the well-being and future of the child if the child is a girl. This may lead to depression as found in our study and it has been reported by many authors also. Gupta et al,[18] also reported higher incidence of PPD in women having previous girl child (OR 3.63, 95%CI 1.51-8.71, P value <0.001) and pressure for male child (OR 2.64, 95%CI 1.1-6.28, P value 0.02). Similar to our study, some researchers did not find any association between gender of the child and postpartum depression.[25]

Postpartum period is a special period in the reproductive life of women as they go through a lot of changes - physically, mentally and socially. Women may find it difficult to adjust to the growing demands and added responsibilities of childbirth. To help her cope in these situations she looks for support from her husband, family and friends, which can be a predictor of postpartum depression. In our research, we found a significant association between postpartum depression and support of husband and family members ($p<0.05$). Similar findings have been corroborated by Rich Edwards et al,[32] child (OR 12.20, 95%CI 3.84-39.82, P value<0.001), Chandran et al and Gausia et al.[33,34]

We also tried to find out if any adverse event in the preceding one year before time of assessment/interview predicted risk of depression. Some of them reported adverse events like accident of mother-in-law, accident of a family member, death of father in-law, death of child. We observed adverse life events to be a significant risk factor associated with depression. As the women is already under stress and burden of childcare, any adverse event might increase the responsibilities of a woman towards her family which might cause psychological stress leading to postpartum depression. Similar findings have been reported by Chandran et al.[32] In fact, Paykel et al,[24] has observed recent stressful life events as the single most important risk factor for puerperal depression (OR 2.10, 95%CI 1.324-5.186, P value 0.039). Silva et al20 found that stressful life events are an important risk factor for the development of postpartum depression though not statistically significant.

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In our study low self-esteem was significantly associated with PPD (P value <0.001). Similar findings were observed by Gokhle et al,[16] (p value0.02) and Steward et al.[35]

There is no previous study associating placental estrogen receptor gene expression and postpartum depression. Although numerous studies are available, which suggest that fall in the estrogen levels in blood after delivery is significantly associated with postpartum depression, about which we have already discussed in review. There was a study conducted by Kugaya A et al in 2003 which concluded that 5- HT 2A receptor binding was significantly increased after estrogen replacement therapy in the right prefrontal cortex (right precentral gyrus [Brodmann's area 9], inferior frontal gyrus [Broadmann's area], medial frontal gyrus [Brodmann's area] and the anterior cingulate cortex [Brodmann's area]). Thus, we can say that estrogen has a role in PPD through serotonin signaling. Estrogen is involved in the regulation of serotonin receptor number and function also.[10] That is why fluctuating estrogen levels across the women's reproductive life have been associated with depressed mood.

Osterlund M K et al,[36] (1999) conducted a study in rats and observed a link between estrogen and serotonin 5-HT in depression. The mRNA levels of the estrogen receptor ER α & β subtypes and the 5-HT1A and 5-HT2A receptors were analyzed in limbic areas of ovariectomized rats treated with 17b-estradiol. In many areas, estradiol was found to regulate the 5-HT receptor mRNA expression.

Ahokas A et al (2001) established the association between estradiol and postpartum depression and found that postpartum depression symptoms were associated with estradiol deficiency and symptoms reduced on treatment with 17 betaestradiol. Estradiol acts through ESR receptors thus ESR receptors are involved in the pathway of depression.[37]

RH Segman et al (2009) conducted a case control study by sampling blood from mothers shortly after delivery and compared it with peripheral blood mononuclear cells (PBMC) gene expression profiles between postpartum depression women and normal postpartum women. A differential gene expression signature was observed in postpartum depression women. The differentially expressed transcripts showed a high number (29 out of 73) of transcripts linked to estrogen signaling.[38]

Mehta D et al (2013) also used peripheral blood gene expression profiles in a high risk, longitudinal cohort. In the first and third pregnancy trimesters and early postpartum of 201 samples, gene expression was measured on Illumina Human HT12 v4 microarrays & plasma estradiol and estriol were measured. 116 transcripts were differentially expressed between the PPD and euthymic women during the third trimester that allowed prediction of PPD with an accuracy of 88%. Within these transcripts, significant enrichment of transcripts implicated that women with PPD displayed an increased sensitivity to estrogen signaling, confirming the previously proposed hypothesis of increased sex steroid sensitivity as a susceptibility factor for PPD. These results suggest that PPD can be robustly predicted in currently euthymic women as early as the third trimester and these findings have implications for predictive testing of high-risk women and prevention and treatment for PPD.[39]

There was a pilot study conducted in 2013 in University of California by Shareen Y. El-Ibiary et al,[40] to assess the influence of genetic and environmental risk factors upon PPD 88. They recruited the patients having Edinburgh Postnatal Depression Score > 14 and <7 for cases and controls. At follow up venous blood samples were withdrawn and gene analysis was done. In the study they selected 12 genes for analysis, including those involved with steroid hormone function (NR3C1, FKBP5, ESR1, ESR2, PGR, AR, AKR1C2), neurotransmitter function (SLC6A4, MAOA, COMT, HTR2A), and neurotrophin function (BDNF). As expected strongest association was found between serotonin 2A receptor (HTR 2A) variants and PPD. They also found some association between estrogen receptor ESR 1 variant (two variants rs 11155820 and 2273206 were studied) and PPD.

CONCLUSION

So we assumed that after delivery as estrogen levels fall (which is already proven), there will be less mRNA production and less translation to protein which will lead to less expression of ESR receptors. And this is proven in our study as ESR α gene in placental tissue was found to be significantly down regulated by 1.33 times among cases as compared to controls.

REFERENCES

1. ICD-10 Version: 2010. Available from: http://apps.who.int/classifications/ icd10/ browse/2010/en#/F53.0. Accessed on 17th April, 2017.
2. Postpartum Depression from Pregnancy Guide, by Peter J. Chen,at Hospital of the University of Pennsyvania. Available from https://en.wikipedia.org/wiki/ Postpartum_depression. Accessed on 2nd April, 2017
3. Yonkers KA, Ramin SM, Rush AJ, Navarrete CA, Carmody T, March D et al. Onset and persistence of postpartum depression in an inter-city maternal health clinic system. Am J Psychiatry 2001;158(11):1856-63.
4. Canadian Mental Health Association. Postpartum Depression. Available from: http://www.cmha.ca/mental_health/postpartum-depression.Accessed on 10th April, 2017
5. Halbreich U, Karkun S. Cross-cultural and social diversity of prevalence of postpartum depression and depressive symptoms. J Affect Disord 2006;91:97-111.
6. Gupta S, Kishore J, Mala YM, Ramji S, Aggarwal R. Postpartum depression in north Indian women: prevalence and risk factors. J Obstet Gynaecol India 2013;63(4):223-9.
7. Lokuge S, Frey BN, Foster JA, Soares CN, Steiner M. Depression in women: windows of vulnerability and new insights into the link between estrogen and serotonin. J Clin Psychiatry 2011;72(11):e1563-9.
8. World Health Organization. Maternal Mental Health & Child Health and Development. Literature review of risk factors and

ISSN (O): 2687-5365; ISSN (P): 2753-6556

intervention of postpartum depression. Department of Mental Health and Substance Abuse. Geneva 2008.Available from www.who.int/mental_health/prevention/suicide/mmh%26chd_chapter_1.pdf.Accessed on October 12, 2015.
9. Mokrani MC, Duval F, Crocq MA, Bailey P, Macher JP. HPA axis dysfunction in depression: correlation with monoamine system abnormalities. Psycho neuroendocrinology 1997;22 Suppl 1:S63-8.
10. Andrade TG, Nakamuta JS, Avanzi V, Graeff FG. Anxiolytic effect of estradiol in the median raphe nucleus mediated by 5-HT1A receptors. Behav Brain Res 2005;163(1):18-25.
11. Bukovsky A, Cekanova M, Caudle MR, Wimalasena J, Foster JS, Henley DC, et al. Expression and localization of estrogen receptor-alpha protein in normal and abnormal term placentae and stimulation of trophoblast differentiation by estradiol. Reprod Biol Endocrinol 2003;1:13.
12. Beck CT. Predictors of postpartum depression: an update. Nurs Res2001;50(5):275-85.
13. Banerjee N, Banerjee A, Kriplani A, Saxena S. Evaluation of postpartum depression using the Edinburgh Postnatal Depression Scale in evaluation of postpartum depression in a rural community in India. Int J Soc Psychiatry 2000:46(1):74-5.
14. Kok L, Chan P, Ratnam S. Postnatal depression in Singapore women. Singapore Med J 1994;35(1):33-5.
15. Affonso DD, De AK, Horowitz JA, Mayberry LJ. An international study exploring levels of postpartum depressive symptomatology. J Psychosom Res 2000;49(3):207-16.
16. Gokhale AV, Vaja A. Screening for postpartum depression. Gujarat Med J 2013;68(2):46-51.
17. Patel HL, Ganjiwale JD, Nimbalkar AS, Vani SN, Vasa R, Nimbalkar SM. Characteristics of Postpartum Depression in Anand District, Gujarat, India. J Trop Pediatr 2015;61(5):364-9.
18. Gupta S, Kishore J, Mala YM, Ramji S, Aggarwal R. Postpartum depression in north Indian women: prevalence and risk factors. J Obstet Gynaecol India 2013;63(4):223-9.
19. Spitzer RL, Kroenke K, Williams JB. Validation and utility of a self-report version of PRIME-MD: the PHQ primary care study. Primary Care Evaluation of Mental Disorders. Patient Health Questionnaire. JAMA 1999;282(18):1737-44.
20. Silva R, Jansen K, Souza L, Quevedo L, Barbosa L, Moraes I, et al. Sociodemographic risk factors of perinatal depression: a cohort study in the public health care system. Rev Bras Psiquiatr 2012;34(2):143-8.
21. Mumford DB, Saeed K, Ahmad I, Latif S, Mubbashar MH. Stress and psychiatric disorder in rural Punjab. Br J Psychiatry 1997;170:473-8.
22. Rahman A, Iqbal Z, Harrington R. Life events, social support and depression in childbirth: perspectives from a rural community in the developing world. Psychol Med 2003;33(7):1161-7.
23. Ghosh A, Goswami S. Evaluation of post partum depression in a tertiary hospital. J Obstet Gynaecol India 2011;61(5):528-30.
24. Paykel ES, Emms EM, Fletcher J, Rassaby ES. Life events and social support in puerperal depression. Br J Psychiatry 1980;136(4):339-46.
25. Mahmud WMRW, Shariff S, Yaacob MJ. Postpartum depression: a survey of the incidence and associated risk factors among malay women in Beris Kubor Besar, Bachok, Kelantan. Malays J Med Sci 2002;9(1):41-8.
26. Ho-Yen SD, Bondevik GT, Eberhard-Gran M, Bjorvatn B. Factors associated with depressive symptoms among postnatal women in Nepal. Acta Obstet Gynecol Scand 2007;86(3):291-7.
27. Mayberry LJ, Horowitz JA, Declercq E. Depression symptom prevalence and demographic risk factors among U.S. women during the first 2 years postpartum. J Obstet Gynecol Neonatal Nurs 2007;36(6):542-9.
28. Nielsen Forman D, Videbech P, Hedegaard M, Dalby Salvig J, Secher NJ. Postpartum depression: identification of women at risk. BJOG 2000;107(10):1210-7.
29. Dorheim SK, Bjorvatn B, Eberhard-Gran M. Can insomnia in pregnancy predict postpartum depression? A longitudinal, population-based study. PLoS One 2014;9(4):e94674.
30. Torkan B, Parsay S, Lamyian M, Kazemnejad A, Montazeri A. Postnatal quality oflife in women after normal vaginal delivery and caesarean section. BMC Pregnancy Childbirth. 2009;9:4.
31. Mazaheri MA, Rabiei L, Masoudi R, Hamidizadeh S, Nooshabadi MR, Najimi A. Understanding the factors affecting the postpartum depression in the mothers of Isfahan city. J Educ Health Promot 2014;3:65.
32. Rich-Edwards JW, Kleinman K, Abrams A, Harlow BL, McLaughlin TJ, Joffe H, et al. Sociodemographic predictors of antenatal and postpartum depressive symptoms among women in a medical group practice. J Epidemiol Community Health 2006;60(3):221-7.
33. Chandran M, Tharyan P, Muliyil J, Abraham S. Post-partum depression in a cohort of women from a rural area of Tamil Nadu, India: Incidence and risk factors. Br J Psychiatry 2002;181(6):499-504.
34. Gausia K, Fisher C, Ali M, Oosthuizen J. Magnitude and contributory factors of postnatal depression: a community-based cohort study from a rural subdistrict of Bangladesh. Psychol Med 2009;39(6):999-1007.
35. Steward D, Robertson E, Dennis C, Grace S, Wallington T. Postpartum depression: Literature review of risk factors and interventions. Toronto: University Health Network Women's Health Program, 2003.
36. Osterlund MK, Overstreet DH, Hurd YL. The flinders sensitive line rats, a genetic model of depression, show abnormal serotonin receptor mRNA expression in the brain that is reversed by 17beta-estradiol. Brain Res Mol Brain Res 1999;74(1-2):158-66.
37. Ahokas A, Kaukoranta J, Wahlbeck K, Aito M. Estrogen deficiency in severe postpartum depression: successful treatment with sublingual physiologic 17 betaestradiol: a preliminary study. J Clin Psychiatry 2001;62(5):3326.
38. Segman RH, Goltser-Dubner T, Weiner I, Canetti L, Galili-Weisstub E, Milwidsky A, et al. Blood mononuclear cell gene expression signature of postpartum depression. Mol Psychiatry 2010;15(1):93-100.
39. Mehta D, Newport DJ, Frishman G, Kraus L, Rex-Haffner M, Ritchie JC, et al. Early predictive biomarkers for postpartum depression point to a role for estrogen receptor signaling. Psychol Med 2014;44(11):2309-22.
40. El-Ibiary SY, Hamilton SP, Abel R, Erdman CA, Robertson PA, Finley PR. A pilotstudy evaluating genetic and environmental factors for postpartum depression. Innov Clin Neurosci 2013;10(9-10):15-22.

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