Iranian Journal of Basic Medical Sciences

ijbms.mums.ac.ir

IJBMS

Effect of maternal fluoxetine exposure on lung, heart, and kidney development in rat neonates

Razieh Taghizadeh Ghavamabadi [1, 2], Zahra Taghipour [1, 2] *, Mahsa Hassanipour [1, 3], Marzieh Khademi [2], Mehdi Shariati [1, 2]

[1] Physiology-Pharmacology Research Center, Rafsanjan University of Medical Sciences, Rafsanjan, Iran
[2] Department of Anatomy, School of Medicine, Rafsanjan University of Medical Sciences, Rafsanjan, Iran
[3] Department of Physiology and Pharmacology, School of Medicine, Rafsanjan University of Medical Sciences, Rafsanjan, Iran

ARTICLE INFO

Article type:
Original article

Article history:
Received: Oct 26, 2017
Accepted: Jan 3, 2018

Keywords:
Fluoxetine
Heart
Kidney
Lung
SSRIs

ABSTRACT

Objective(s): Depression during pregnancy negatively affects fetal development. Fluoxetine as a selective serotonin reuptake inhibitor (SSRIs) is used for treatment of gestational depression. This study is trying to determine the effects of fluoxetine on the renal, heart and lung development.

Materials and Methods: Fifteen pregnant rats were treated with fluoxetine at 7 mg/kg from days 0 to 21 of gestation. Immediately after born, heart and kidney samples were evaluated for genes expression and histological assessment. Lung sample were fixed for immunohistochemical study.

Results: The gene expression of BMP7 and WNT4 were reduced in the kidney of fluoxetine-treated group (*P*-value<0.05), but in the heart of both groups no significant difference was found in gene expression (*P*-value>0.05). Histological assessment showed that the glomeruli of the kidneys in treated group are more primordial compared to control. There was a developmental deficiency in Bowman's capsule, and the capsular space was not clear. The arrangements of the filaments, the position of the nucleus and cells morphology were normal in the hearts of both groups. Immunohistochemical analysis demonstrated that in the fluoxetine-exposed group HoxB5 is more expressed in the mesenchymal cells, but in the control group the expression is limited to alveolar cells.

Conclusion: According to developmental changes in kidney, heart and lung, fluoxetine affects neonatal growth during pregnancy, which may lead to delay of some organs growth. So, it is essential to survey the roles of antidepressant drugs on fatal and neonatal development during pregnancy.

► *Please cite this article as:*
Taghizadeh Ghavamabadi R, Taghipour Z, Hassanipour M, Khademi M, Shariati M. Effect of maternal fluoxetine exposure on lung, heart and kidney development in rat neonates. Iran J Basic Med Sci 2018; 21:417-421. doi: 10.22038/IJBMS.2018.27203.6650

Introduction

The emotional state during pregnancy is an important aspect in medicine. Pregnancy enhances the vulnerability for depression onset or return (1). Depression, anxiety and other mood disorders are associated with obstetric, fetal and neonatal outcomes such as impaired cognition and attention deficit hyperactivity disorder (2, 3). As untreated maternal depression has serious health impact, a rational pharmacotherapy is of great importance.

Selective serotonin reuptake inhibitors (SSRIs) have been studied for antepartum depression based on the severity of condition (4). Soon after SSRIs introduction (1988) and their efficacy in treatment of pregnancy-related mood disorders, the studies reported adverse neonatal signs (5, 6). SSRI therapy has been proposed to have a link with neurobehavioral disturbances, preterm birth, lower birth weight, neurotoxic effects and behavioral teratogenic effects, cardiac malformation, pulmonary hypertension, movement disorders and convulsion (7-9).

Serotonin, a key signaling molecule in progenitor heart cells, is involved in development of the outflow tract, myocardial cell differentiation, and separation of the heart chambers; therefore, administration of serotonin reuptake inhibitors during pregnancy can stimulate defective heart morphogenesis (10). Some studies have demonstrated that maternal exposure to fluoxetine in early pregnancy was associated with cardiac malformation and congenital heart defects (10, 11), while some studies have shown that there is no linkage between SSRIs and congenital heart defects (12, 13). Studies have also shown that fluoxetine can lead to ventricular septal defects (14) and atrial septal defects (15).

Several studies demonstrated that SSRIs induce hyponatremia in adult (16-18). No significant difference exists between SSRI members, but one study indicated that fluoxetine, citalopram and citalopram exert higher effects on this disorder than other SSRIs (19). Some studies reported the correlation between hyponatremia and the use of fluoxetine. These studies explained that fluoxetine enhances water permeability, which leads to renal water absorption (a cause of hyponatremia) (20-22). Renal dysplasia can also be a result of using SSRIs, principally fluoxetine (23).

Our previous study showed that exposure to fluoxetine during pregnancy can lead to a delay in lung development (24). In that study, HoxB5 and SPC were evaluated as genes of the alveolar epithelium. Increasing of HoxB5 expression based on real-time polymerase chain reaction (PCR) test and histological analyzes demonstrated that this gene expresses in the mesenchymal cells and not in the alveolar type I cells, but it was essential to confirm the expression of HoxB5 and SPC by immunohistochemistry method. Kidney and heart are mesodermal tissues and based on the reports showing the correlation between these two tissues and

**Corresponding author:* Zahra Taghipour. Department of Anatomy, School of Medicine, Physiology-Pharmacology Research Center, Rafsanjan University of Medical Sciences, Rafsanjan, Iran. Tel: +98-3431315064; Email: taghipourz@yahoo.com

fluoxetine, we also evaluated the impact of fluoxetine on heart and renal development.

In the developmental process of heart, Foxp1 gene is expressed during the early stage of development, while Foxc1 and Foxc2 genes are expressed in final stages during formation of four chambered heart (25).

WT1 gene plays a role in renal glomerular podocyte differentiation and is effective in expression of podocyte markers (26). GDNF is critical for signaling and directing ureteric bud growth and its reduction results in ureter budding limitation in the metanephric tissue. WNT4 and BMP7 genes have a role in development of metanephric mesenchyme. WNT4 is important for epithelium formation in nephrons, and BMP7 is required for the metanephric condensates, comma- and S-shaped bodies (27). Therefore, in the present study we completed our previous findings on the lung tissues and examined the heart and renal development in fluoxetine-exposed rat newborns.

Materials and Methods

Thirty female Wistar rats weighing 200–250 g and aging 4-5 months were purchased from animal house of Rafsanjan University of Medical Sciences and were kept under controlled conditions at 23 ˚C with free access to sufficient food and water and a constant 12 hr light/12 hr dark cycle. Every three female rat were placed in contact with an adult male rat for mating. After 24 hr, vaginal smears were evaluated in female rats. On the day of sperm detection in vaginal smear (gestation day 0), the female rats were randomly separated into treatment and control groups. The treatment group was treated by gastric gavage with fluoxetine at 7 mg/kg once per day (24) from days 0 to 21 of gestation. The control rats received a similar volume of distilled water. Immediately after born, lung, heart and renal samples were separated from newborns. Then, some hearts and right kidneys were fixed in TRizol reagent for real-time PCR and some hearts, left kidneys and lung samples were fixed in 4% paraformaldehyde for histological analysis.

Real-time PCR

Total RNA was extracted from the heart and kidney tissues using TRizol reagent according to the manufacturer's protocol. Extracted RNA was purified by isolation kits and used as a template for reverse transcription in cDNA synthesis. Real-time PCR was undertaken for Foxc1, Foxc2 and Foxp1 genes in heart samples and WT1, GDNF, WNT4 and BMP7 genes in kidney samples, and β-actin (housekeeping gene) genes in triplicate. Designed primers for each gene were controlled thermodynamically and then they were evaluated in the BLAST database to verify the absence of nonspecific binding to other regions of the genome. The sequences of used primers were described in Table 1.

Real-time PCR was performed using program on a Bio-Rad CFX96 system (Bio-Rad Laboratories Inc., Hercules, CA, USA). The relative quantification of PCR products was determined using the $2^{-\Delta Ct}$ formula (28). The melting curves, quantitative analyses, and dissociation stages of the data were performed using the CFX manager software.

Histological and morphological assessment

The samples of heart and kidney that had been fixed in formalin were embedded in paraffin, and then the tissues were sectioned at 8 µm by microtome and were stained with hematoxylin and eosin. The samples were studied under a light microscope (Olympus BX51) equipped with camera (Olympus DP25) and Cell software.

Table1. The sequence of primers used for real-time polymerase chain reaction in the study

Gene	Forward primer	Reverse primer
FoxC1	ACCATGGCTATCCAGAATGC	GTCCCGATAGAAGGGAAAGC
Foxc2	AGCATCACAGTCACCTCCAC	TGCGAGTTGAACATCTCCCG
Foxp1	ATGAACCCACACGCCTCTAC	GTTTTAGAAAGGCCGGGAAG
BMP7	GAGGGCTGGTTGGTATTTGA	AACTTGGGGTTGATGCTCTG
WNT4	ACTGGACTCCCTGCCTGTCTT	GTCCGGTCACAGCCACACTT
WT1	GCCTTCACCTTGCACTTCTC	GACCGTGCTGTATCCTTGG
GDNF	TCACTGACTTGGGTTTGGGC	AACATGCCTGGCCTACCTTG
β-actin	GGGCATGGGTCAGAAGGATT	CGCAGCTCATTGTAGAAGGT

Immunohistochemical evaluation

Four lung paraffin-embedded tissues of each group were selected. Then, they were sectioned serially at 6 µm. Three serial sections were selected for immunostaining using Trypsin Antigen Retrieval Protocol. The first tissue sections were deparaffinized by xylene and hydrated by a graded series of ethanol and then rinsed in distilled water. The sections were covered with trypsin working solution and incubated for 20 min at 37 ˚C in humidified chamber. After that, they were kept at room temperature for 10 min and they were rinsed in phosphate-buffered saline (PBS) with Tween 20 and were blocked in GSA and Tris-buffered saline (TBS) for 2 hr. Some tissue sections were incubated with HoxB5 antibody (Bioss Company) at a 1:150 concentration and some tissue sections were incubated with SPC antibody (Bioss Company) at a 1:150 concentration overnight at 4 ˚C. After rinsing the slides by TBS with Triton X-100 and H_2O_2 in PBS, the slides were incubated with Goat Anti-Rabbit IgG (Abcam Company) (1:1000 concentration) for 1 hr at room temperature, then they were stained with DAB 3% for 10 min and after rinsing were counterstained with hematoxylin. The stained slides were studied under a light microscope (Olympus BX51) equipped with camera (Olympus DP25) and Cell software.

Statistical analysis

All data were offered as means±SEM and were analyzed using GraphPad Prism 5.04. The analysis of differences between groups were performed with a t test and data measured as statically significant at *P*-value<0.05.

Results

Real-time PCR analysis in kidney

The expression of kidney related genes (WT1, GDNF, BMP7 and WNT4) in both groups has been shown in Figure 1. The expression of GDNF, BMP7 and WNT4 genes were reduced compared to control group and this reduction for BMP7 and WNT4 was significant (*P*-value <0.05), while the reduction of GDNF was not significant (*P*-value >0.05). The comparison between fluoxetine-exposed group and control group showed enhancement in the expression of WT1, but it was not statistically significant (*P*-value >0.05).

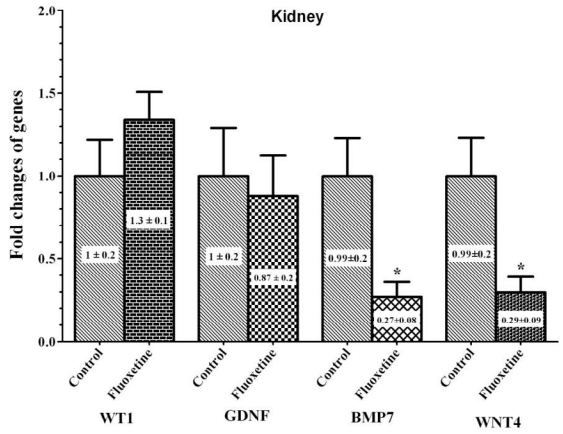

Figure 1. T test for statistical analysis of genes expression in the kidney. There was no difference in the expression of WT1 and GDNF genes between kidneys of fluoxetine - treated group and control group (P-value: 0.08 and 0.87, respectively). The expression of BMP7 and WNT4 genes in kidney of fluoxetine - treated group reduced significantly compared to control group (*P*-value: 0.005 and 0.01, respectively)

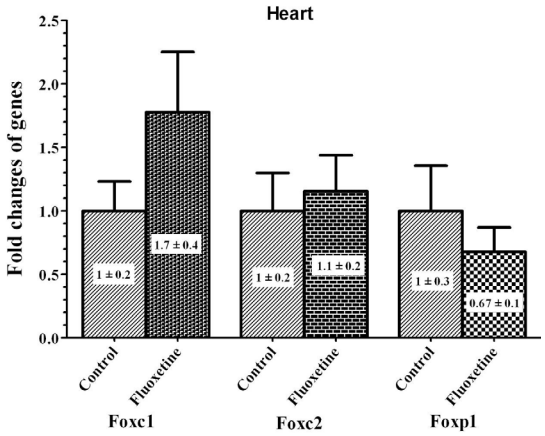

Figure 2. T test for statistical analysis of genes expression in the heart. The expression of Foxc1 and Foxc2 genes increased in hearts of fluoxetine - treated group compared to control group, but this increase was not significant (P-value: 0.41 and 0.61, respectively). The expression of Foxp1 reduced in hearts of fluoxetine - treated group compared to control group, but this reduction was not significant (*P*-value: 0.41)

Real-time PCR analysis in heart

Figure 2 shows the expression of Foxc1, Foxc2 and Foxp1 genes in the heart of both groups. Our analysis did not show any significant difference between the expression of Foxc1, Foxc2 and Foxp1 in fluoxetine-treated group and control group.

Immunohistochemical analysis

The genes that are involved in lung development are HoxB5 and SPC. Therefore, in this study we evaluated the expression of HoxB5 and SPC in control and fluoxetine-exposed lung samples. SPC is a cytoplasmic gene. In the fluoxetine-exposed group, the expression of SPC was not different with control group (Figure 3A, B). In the earliest stages of lung development, HoxB5 is expressed in the mesenchymal cells and is a nuclear marker. In the fluoxetine-exposed group, the black nuclei, markers of HoxB5, were observed in the mesenchymal cells, but in the control group they were limited to alveolar cells and it seems that the mesenchymal cells in the fluoxetine-exposed group are more than control group (Figure 3C, D).

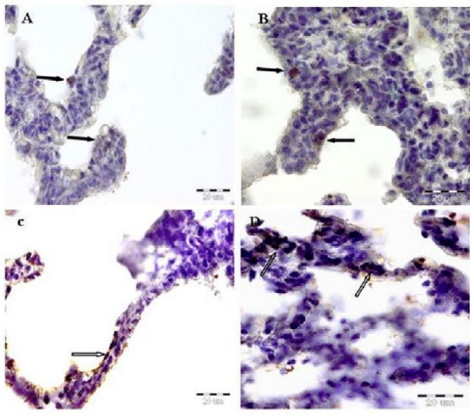

Figure 3. A, B: The expression of SPC gene in alveolar cells of lung in fluoxetine - treated and control groups, respectively. Brown stain in cytoplasm shows SPC expression (Arrows).
C, D: The expression of HoxB5 gene in fluoxetine-treated and control groups. Nucleus of HoxB5 positive cells is dark (arrows), but other nucleus are blue. C; HoxB5 positive cells in control group are located in alveolar epithelium (arrow). But, these cells in fluoxetine - treated group are found in both mesenchymal and alveolar cells (D).
(Hematoxylin and Eosin stain. Magnification ×100 (scar bar: 20 µm))

Histological assessment

The kidney of control and treated groups has been shown in the Figure 4. In the control group, the metanephric tissue is more developed, glomeruli are more orderly and glomerular capsular space is completely clear. Epithelium of parietal layer of Bowman's capsule is squamous and regular.

In the treated group, the glomeruli are more primordial and are not coherent, and it seems that there is not enough development in Bowman's capsule, and also the glomerular capsular space could not be observed well.

Figure 5 shows the heart muscle cells in control and fluoxetine-exposed groups. In both groups, arrangements

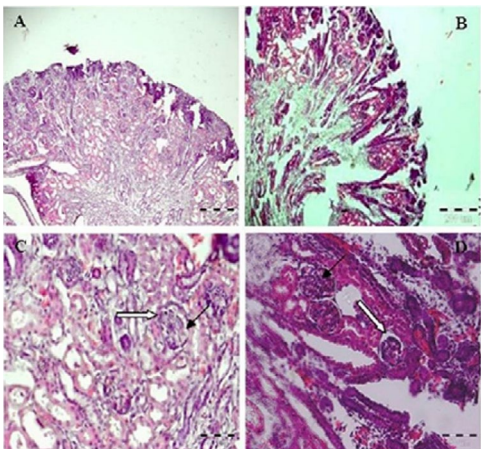

Figure 4. A, C: Kidney of fluoxetine - treated group. B, D: Kidney of control group.
Arrow: Glomerular capsular space. The glomerular capsular space is clear in the control group
(Hematoxylin and Eosin stain. Magnification of A, B ×4 (scar bar: 200 µm), Magnification of C, D ×40 (scar bar: 50 µm)

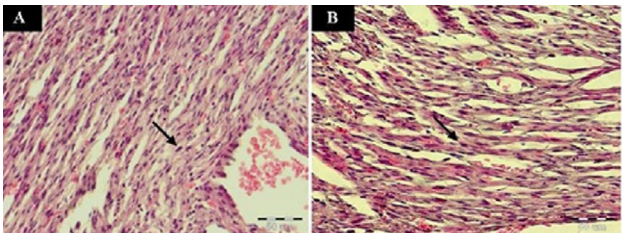

Figure 5. A: Heart of control group. B: Heart of fluoxetine - treated group Arrow: The nuclei of cardiac cells. (Hematoxylin and Eosin stain. Magnification ×40 (scar bar: 50 µm)

of the filaments, the position of the nucleus and cell morphology are normal.

Discussion

The present study investigated the effects of fluoxetine on the lung, renal and heart development. For this purpose, the expression of WT1, GDNF, BMP7 and WNT4 genes were evaluated to study the development of kidney. During the development, kidney is derived from the ureteric bud and the metanephric mesoderm. GDNF gene is responsible for the ureteric bud growth (29). BMP7 and WNT4 are expressed in metanephric mesoderm development. WT1 has been considered as an inducer of podocytes development (27). So, we evaluated the expression of the abovementioned genes in this experiment.

The results exhibited that there is no difference between the two groups in expression of GDNF; however, the expression of BMP7 and WNT4 showed significant reduction compared to control group. This effect was consistent with our histological results. The data revealed that in the fluoxetine-exposed group, the development of metanephric tissue was less than the control group. Findings have shown that fluoxetine can change the serum sodium level, which leads to hyponatremia in the kidney (17, 23). Studies indicated that using SSRIs in early pregnancy may be associated with cystic kidney or kidney agenesis (30-32)

It has been reported that SSRIs, especially fluoxetine and paroxetine, could result in the enhancement of congenital heart defects (11, 33, 34), while some other studies did not show any relation between SSRIs and heart malformations (14, 32, 35). Nembhard *et al.* showed that the use of SSRI during early pregnancy leads to metabolic pathway dysfunction and an increase in oxidative stress level leading to formation of the harmful free radicals, which are damaging to the developing cardiac tissue (36). Two separate studies explored the effects of fluoxetine or paroxetine on the heart and reported the atrial septal abnormality and right ventricular outflow tract obstruction defect (37-39). Evidences showed that SSRIs can result in ventricular and atrial septum defects (31, 39-41). Studies reported that SSRIs can affect the development of the heart through changes in serotonin transporter and prenatal 5-HT levels. SSRIs inhibit the expression of serotonin transporter in the embryonic cardiac cells and thereby reduce serotonin in the cells. The reduction of serotonin disturbs the normal cardiac development (42). Kaihola *et al.* studied the fetal development in the presence of fluoxetine and showed that fluoxetine has some effects on the timing of developmental stages (43).

In this study, we did not observe any alternation in heart development of the fluoxetine – exposed group by real-time-PCR and histological analysis, but the gene expression of the final stage in heart development (Foxc1, Foxc2), related to chambering stage, were increased in treated group in comparison with control group; however, this difference was not statistically significant.

As regards to increasing of Foxc1 and Foxc2 genes expression, the development of heart in fluoxetine-exposed neonates may occur more quickly, which may lead to septal defects, but more studies are need to clarify the role of SSRIs on fetus heart development.

Based on our previous study, HoxB5 gene showed an overexpression with real-time-PCR in lung of fluoxetine – exposed neonates. In this study, the expression of HoxB5 in lung mesenchymal cells confirmed the results of the previous study (24). Although HoxB5 in both groups of control and treatment is expressed in alveolar type I cells, the expression of this gene is also observed in mesenchymal cells in fluoxetine-exposed group. There is no significant difference in expression of SPC between control and fluoxetine groups, which demonstrates a lack of differentiation in mesenchymal tissue.

Conclusion

The result of this study showed that crossing of the fluoxetine from the placenta could exert adverse effects on lung and renal development and leads to a delay in development of these organs. Our data did not exhibit any significant change in cardiac tissue and related genes. According to the result of this study, it is essential to survey the roles of antidepressants on fetus during pregnancy.

Acknowledgment

This work was supported by Rafsanjan University of Medical Sciences, Kerman, Iran.

References

1. Bennett HA, Einarson A, Taddio A, Koren G, Einarson TR. Prevalence of depression during pregnancy: systematic review. Obstet Gynecol 2004; 103:698-709.
2. Alder J, Fink N, Bitzer J, Hosli I, Holzgreve W. Depression and anxiety during pregnancy: a risk factor for obstetric, fetal and neonatal outcome? A critical review of the literature. J Matern Fetal Neonatal Med 2007; 20:189-209.
3. Ryan D, Milis L, Misri N. Depression during pregnancy. Can Fam Physician 2005; 51:1087-1093.
4. Chatillon O, Even C. [Antepartum depression: prevalence, diagnosis and treatment]. Encephale 2010; 36:443-451.
5. Chambers CD, Johnson KA, Dick LM, Felix RJ, Jones KL. Birth outcomes in pregnant women taking fluoxetine. N Engl J Med 1996; 335:1010-1015.
6. Moses-Kolko EL, Bogen D, Perel J, Bregar A, Uhl K, Levin B, *et al.* Neonatal signs after late in utero exposure to serotonin reuptake inhibitors: literature review and implications for clinical applications. JAMA 2005; 293:2372-2383.
7. Costei AM, Kozer E, Ho T, Ito S, Koren G. Perinatal outcome following third trimester exposure to paroxetine. Arch Pediatr Adolesc Med 2002; 156:1129-1132.
8. Kallen B. Neonate characteristics after maternal use of antidepressants in late pregnancy. Arch Pediatr Adolesc Med

2004; 158:312-316.
9. Oberlander TF, Warburton W, Misri S, Aghajanian J, Hertzman C. Neonatal outcomes after prenatal exposure to selective serotonin reuptake inhibitor antidepressants and maternal depression using population-based linked health data. Arch Gen Psychiatry 2006; 63:898-906.
10. Wemakor A, Casson K, Garne E, Bakker M, Addor MC, Arriola L, *et al.* Selective serotonin reuptake inhibitor antidepressant use in first trimester pregnancy and risk of specific congenital anomalies: a European register-based study. Eur J Epidemiol 2015; 30:1187-1198.
11. Colvin L, Slack-Smith L, Stanley FJ, Bower C. Dispensing patterns and pregnancy outcomes for women dispensed selective serotonin reuptake inhibitors in pregnancy. Birth Defects Res A Clin Mol Teratol 2011; 91:142-152.
12. Ban L, Gibson JE, West J, Fiaschi L, Sokal R, Smeeth L, *et al.* Maternal depression, antidepressant prescriptions, and congenital anomaly risk in offspring: a population-based cohort study. BJOG 2014; 121:1471-1481.
13. Wang S, Yang L, Wang L, Gao L, Xu B, Xiong Y. Selective Serotonin Reuptake Inhibitors (SSRIs) and the Risk of Congenital Heart Defects: A Meta-Analysis of Prospective Cohort Studies. J Am Heart Assoc 2015; 4:e001681.
14. Malm H, Artama M, Gissler M, Ritvanen A. Selective serotonin reuptake inhibitors and risk for major congenital anomalies. Obstet Gynecol 2011; 118:111-120.
15. Jimenez-Solem E, Andersen JT, Petersen M, Broedbaek K, Jensen JK, Afzal S, *et al.* Exposure to selective serotonin reuptake inhibitors and the risk of congenital malformations: a nationwide cohort study. BMJ Open 2012; 2:e001148.
16. Matsumoto H. Hyponatremia associated with selective serotonin reuptake inhibitors. Intern Med 2005; 44:173-174.
17. Shakibaei F, Gholamrezaei A, Alikhani M, Talaeizadeh K. Serum sodium changes in fluoxetine users at different age groups. Iran J Psychiatry 2010; 5:113-116.
18. Viramontes TS, Truong H, Linnebur SA. Antidepressant-Induced Hyponatremia in Older Adults. Consult Pharm 2016; 31:139-150.
19. Carvalho AF SM, Brunoni AR, Vieta E, Fava GA. The safety, tolerability and risks associated with the use of newer generation antidepressant drugs: a critical review of the literature. Psychother Psychosom 2016; 85:270-288.
20. Girault C, Richard JC, Chevron V, Goulle JP, Droy JM, Bonmarchand G, *et al.* Syndrome of inappropriate secretion of antidiuretic hormone in two elderly women with elevated serum fluoxetine. J Toxicol Clin Toxicol 1997; 35:93-95.
21. ten Holt WL, van Iperen CE, Schrijver G, Bartelink AK. Severe hyponatremia during therapy with fluoxetine. Arch Intern Med 1996; 156:681-682.
22. Verbalis JG. Disorders of body water homeostasis. Best Pract Res Clin Endocrinol Metab 2003; 17:471-503.
23. Moyses ZP, Nakandakari FK, Magaldi AJ. Fluoxetine effect on kidney water reabsorption. Nephrol Dial Transplant 2008; 23:1173-1178.
24. Taghizadeh R, Taghipour Z, Karimi A, Shamsizadeh A, Taghavi MM, Shariati M, *et al.* The expression of HoxB5 and SPC in neonatal rat lung after exposure to fluoxetine. Drug Des Devel Ther 2016; 10:3323-3329.
25. Zhu H. Forkhead box transcription factors in embryonic heart development and congenital heart disease. Life Sci 2016; 144:194-201.
26. Palmer RE, Kotsianti A, Cadman B, Boyd T, Gerald W, Haber DA. WT1 regulates the expression of the major glomerular podocyte membrane protein Podocalyxin. Curr Biol 2001; 11:1805-1809.
27. Lechner MS, Dressler GR. The molecular basis of embryonic kidney development. Mech Dev 1997; 62:105-120.
28. Schmittgen TD, Livak KJ. Analyzing real-time PCR data by the comparative C(T) method. Nat Protoc 2008; 3:1101-1108.
29. Marcotte M, Sharma R, Bouchard M. Gene regulatory network of renal primordium development. Pediatr Nephrol 2014; 29:637-644.
30. Diav-Citrin O, Ornoy A. Selective serotonin reuptake inhibitors in human pregnancy: to treat or not to treat? Obstet Gynecol Int 2012; 2012:698947.
31. Kallen BA, Otterblad Olausson P. Maternal use of selective serotonin re-uptake inhibitors in early pregnancy and infant congenital malformations. Birth Defects Res A Clin Mol Teratol 2007; 79:301-308.
32. Reis M, Kallen B. Delivery outcome after maternal use of antidepressant drugs in pregnancy: an update using Swedish data. Psychol Med 2010; 40:1723-1733.
33. Knudsen TM, Hansen AV, Garne E, Andersen AM. Increased risk of severe congenital heart defects in offspring exposed to selective serotonin-reuptake inhibitors in early pregnancy--an epidemiological study using validated EUROCAT data. BMC Pregnancy Childbirth 2014; 14:333.
34. Nikfar S, Rahimi R, Hendoiee N, Abdollahi M. Increasing the risk of spontaneous abortion and major malformations in newborns following use of serotonin reuptake inhibitors during pregnancy: A systematic review and updated meta-analysis. Daru 2012; 20:75.
35. Pedersen LH, Henriksen TB, Vestergaard M, Olsen J, Bech BH. Selective serotonin reuptake inhibitors in pregnancy and congenital malformations: population based cohort study. BMJ 2009; 339:b3569.
36. Nembhard WN, Tang X, Hu Z, MacLeod S, Stowe Z, Webber D, *et al.* Maternal and infant genetic variants, maternal periconceptional use of selective serotonin reuptake inhibitors, and risk of congenital heart defects in offspring: population based study. BMJ 2017; 356:j832.
37. Furu K, Kieler H, Haglund B, Engeland A, Selmer R, Stephansson O, *et al.* Selective serotonin reuptake inhibitors and venlafaxine in early pregnancy and risk of birth defects: population based cohort study and sibling design. BMJ 2015; 350:h1798.
38. Reefhuis J, Devine O, Friedman JM, Louik C, Honein MA, National Birth Defects Prevention S. Specific SSRIs and birth defects: Bayesian analysis to interpret new data in the context of previous reports. BMJ 2015; 351:h3190.
39. Daud AN, Bergman JE, Kerstjens-Frederikse WS, Groen H, Wilffert B. The risk of congenital heart anomalies following prenatal exposure to serotonin reuptake inhibitors-is pharmacogenetics the Key? Int J Mol Sci 2016; 17:1333.
40. Kornum JB, Nielsen RB, Pedersen L, Mortensen PB, Norgaard M. Use of selective serotonin-reuptake inhibitors during early pregnancy and risk of congenital malformations: updated analysis. Clin Epidemiol 2010; 2:29-36.
41. Bakker MK, Kerstjens-Frederikse WS, Buys CH, de Walle HE, de Jong-van den Berg LT. First-trimester use of paroxetine and congenital heart defects: a population-based case-control study. Birth Defects Res A Clin Mol Teratol 2010; 88:94-100.
42. Velasquez JC, Goeden N, Bonnin A. Placental serotonin: implications for the developmental effects of SSRIs and maternal depression. Front Cell Neurosci 2013; 7:47.
43. Kaihola H, Yaldir FG, Hreinsson J, Hornaeus K, Bergquist J, Olivier JD, *et al.* Effects of fluoxetine on human embryo development. Front Cell Neurosci 2016; 10:160.