Pilot Study of Biomarkers of Oxidative Stress and Chlamydia trachomatis in Female Patients with Spontaneous Abortions

[1]Maysaa El Sayed Zaki.
[2]Yasser Abd El Dayem,
[1]Clinical Pathology Department,
[2]Gynecology and Obstetrics Department, Mansoura Faculty of Medicine

Abstract

The aims of the present study were i. asses the biomarkers of oxidative stress including superoxide anion radical, Nitrite measurement and reduced glutathione in female patients with spontaneous abortions ii. Asses the prevalence of C. trachomatis antibodies IgA and IgG in those patients, iii. Correlate the levels of the oxidative biomarkers to the presence of C. trachomatis antibodies.

The study included one hundred female patients with 2 or more spontaneous abortions in addition to one hundred healthy control females with normal gravidity and parity history. Blood samples were obtained from each subject and subjected to laboratory determination of superoxide anion radical, Nitrite measurement and reduced glutathione by biochemical methods. Determination of specific immunoglobulin A and G (IgA, IgG) was determined by enzyme linked immunosorbent assay (ELISA).

The prevalence of IgA and IgG for C. trachomatis in patients was 4% and 8% respectively and, in the control, subjects the prevalence of IgA and IgG were 1% and 2% respectively. The concentration of oxidative stress products was significantly higher in the patients group compared to the control group (P=0.0001). The concentrations of nitrite and reduced glutathione were 27.9± 4.7, 31.4± 1.9 nmol/ml respectively in patients and the concentrations of nitrite and reduced glutathione were 18.9± 2.1 and 27.9± 3.9 respectively in the control group. The concentration of SOD was significantly reduced in the patients group compared to the control group (P=0.0001). The concentration of SOD was 55.1 6.9 in the patients and in the control the concentration was 64.5± 7.7 nmol/ml In the study of oxidative stress markers in the patients with positive serology for C. trachomatis, there was significant increase in nitrite concentrations compared to patients negative for C. trachomatis serological markers (P=0.005). While reduced glutathione concentration had insignificant increase in patients positive for C. trachomatis with reduced SOD compared to patients with negative serology to C. trachomatis, P=0.6, P=0.07, respectively.

The present study highlights that there was significant increase in oxidant stress biomarkers nitrite and reduced glutathione with significant reduction in superoxide dismutase in patients with repeated abortions. The prevalence of IgG to C. trachomatis was significantly prevalent in patients with recurrent abortion compared to control subjects. The nitrite was significantly correlated with positive serology to C. trachomatis.

Corresponding author:
Yasser Abd El Dayem,
Gynecology and Obstetrics Department, Mansoura Faculty of Medicine

INTRODUCTION

Infertility is a health problem that affects from 8-12% of the worldwide couples. The main cause in about 70% is associated with female infertility due to ovulation problems or male infertility associated with reduced semen quality. However, up to 30% of infertility has no known etiology (1-3). There are causes of infertility attributed to impaired oocyte growth, maturation and implementation (4).

Among pathophysiological process associated with defective oocyte development is oxidative stress with increase in the reactive oxygen species (ROS), high lipid peroxidation (LPO), and decreased total antioxidant capacity (TAC) in follicular fluid (FF) correlate with poor embryo quality and fertilization rates (5, 6), no consensus has yet been achieved in this matter.

Among pathogenic effects of certain microorganisms that lead to infertility is Chlamydia trachomatis (C. trachomatis) infections in genital tract. Chlamydia trachomatis infection affects upper genital tract in females leading to pelvic inflammatory disease and affect the fallopian tubes with fibrosis and tubal factor infertility (6). There is association between C. trachomatis infection in females and increase production of the products of oxidative stress from activated neutrophils and macrophages. The increase products of the oxidativestress affect DNA with oxidative damage leading to aberrant gene expression and inhibition of protein synthesis (7). In vitro experimental study infection of the cell lines with C. trachomatis leads to release of reactive oxygen species with peroxidation of the lipid (8). The effect of peroxidation assists in the spread of the elementary bodies of C. trachomatis by lysis of the infected cells, thus exaggerating the inflammation which results from infection(9). In addition to these effects,there is oxidative damage of the fallopian tubes which leads to increase of 8-hydroxy-2- deoxyguanosineand results in the damage of DNA (10). Moreover, the biomarker of endogenous oxidative DNA damage, 8-hydroxy-2- deoxyguanosine has been reported to be associated with reduced rate of fertilization and low quality oocytes (11, 12).

From the previous study, there is a suggestion for the interaction between the oxidative stress and the persistence of chlamydial infection in the etiology of female infertility. The mechanisms of this interaction can be a clue for providing a new treatment for those patients and in assisting the prognosis (13).

Therefore the aims of the present study were i. asses the biomarkers of oxidative stress including superoxide anion radical, Nitrite measurement and reduced glutathione in female patients with spontaneous abortions ii. Asses the prevalence of C. trachomatis antibodies IgA and IgG in those patients, iii. Correlate the levels of the oxidative biomarkers to the presence of C. trachomatis antibodies.

Material and Method

Subjects

The study was a case- control study. One hundred female patients with 2 or more spontaneous abortions were recruited from Mansoura University Hospital from January 2019 till January 2020. Moreover, one hundred healthy control females with normal gravidity and parity history were included. The patients were above 18 years old with normal hormone profile and no any associated diseases such as liver disorders, renal disorders or autoimmunity. The study was approved by Mansoura ethical committee and approval consent was obtained from each participant in the study. Each female was subjected to full medical history and clinical examination.

Laboratory Investigation

Ten milliliter of heparinized blood was withdrawn from each female under standard precautions. Sera were separated from each blood sample and subjected to laboratory determination of superoxide anion radical, Nitrite measurement and reduced glutathione by biochemical methods. Then aliquots of the serum was kept frozen at -20C for further determination of C. trachomatis antibodies IgA (RIDASCREEN® R-BiopharmAGAn der neuenBergstraße 17 64297 Darmstadt, Germany Chlamydia trachomatis IgA and IgG (Biovision- 155 S. Milpitas Blvd., Milpitas, CA 95035 USA |) by enzyme linked immunosorbant assay (ELISA).

Determination of Superoxide dismutase reductase (SOD)

The biochemical method used for determination of the concentration of superoxide anion depended upon the reduction of nitroblue- tetrazoliumand the formation ofnitroblue-formazan with the measurement of the intensity of the color by spectrophotometry (14). Ten microliter of nitroblue- tetrazolium was added to 100 microliter of plasma with incubation at 37C for 45 minutes. Then 10 microliter of Dimethyl sulfoxide was added and the intensity of the color was measured by spectrophotometer at 550 nm on microplate reader (). The concentration of superoxide dismutase reductase was reported in nmol/ml.

Nitrite measurement

The concentration of nitrite was determined as an indicator to the level of nitric oxide bytheGriessmethod (15). Serial dilution was performed for standard nitrite solution with 100 mM concentration from 100 to 1.6 mM in triplicate microtiter plate and 100 microliters of Griess reagent composed of equal volumes of 0.1% N-(1-naphthyl) ethylenediamineand 1% sulfanilic acid was added with 50 microliter of plasma samples. After incubation for 5 minute and 10 minute. Measurement was performed by the use of enzyme linked microplate reader at wave length 550 nm (RT-2100C, Rayto, Shenzhen, P.R. China). The results were expressed in nmol/ ml from a standard curve established in each test, constituted of known molar concentrations of nitrites.

Determination of reduced glutathione

The concentration measurement was based upon the oxidation of the reduced form of glutathione with sulphide reagent 5-50-dithiobis-2-nitro-benzoic acid, forming a yellow product of 50-thio-2- nitrobenzoic acid (TNB) (16). The reaction was performed by adding equal volumes of 100 microliter of the samples and sulphosalicylic acid (2.5%) and incubation for 10 minutes. Color reaction was measured by spectrophotometer on microplate reader at 405 nm (). The results were expressed in nmol/ml from a standard curve established in each test, constituted of known molar GSH concentrations.

C.trachomatis Specific IgA

The kit used specific antigen from C. trachomatisouter-membrane-Then substrate will be added for this enzyme which will be converted to blue color. The reaction will be stopped by adding sulphuric acid. The detection of the presence of specific IgA antibodies will be carried out by microplate reader with wave length 450 nm.

Determination of C. trachomatis IgG

The kit used purified Chlamydia trachomatis antigen that was coated on the wells of microplate. In the presence of specific IgG for C. trachomatis in the plasma samples, binding reaction will be formed. Second antibody labeled with enzyme conjugate will be added to bind to the formed complex. Then an enzyme substrate will be added and the reaction will be stopped after incubation. The amount of IgG will be proportional to the intensity of the developed color which will be measured by microplate reader at wave length 450 nm.

Statistical analysis

The data of the results will be analyzed by the use of SPSS24. Quantitative data will be expressed as mean and standard deviation and the comparison will be performed by T test and a nova test. Qualitative data will be expressed as number and percentage and the comparison will be performed by Chi-square. P will be considered significant if >0.05.

Results

The study included 100 patients with spontaneous abortions from 2 up to 4 times with normal hormone profiles with mean age SD 29.9± 5.7. There were also, 100 females with normal parity and gravidity history with mean age SD 29.8± 5.9 years. The prevalence of C. trachomatis IgG in patients was significantly higher in patients (P=0.05) compared to the control subjects. The prevalence of IgA and IgG for C. trachomatis in patients was 4% and 8% respectively and, in the control, subjects the prevalence of IgA and IgG were 1% and 2% respectively. The concentration of oxidative stress products was significantly higher in the patients group compared to the control group (P=0.0001). The concentrations of nitrite and reduced glutathione were 27.9± 4.7, 31.4± 1.9 nmol/ml respectively in patients and the concentrations of nitrite and reduced glutathione were 18.9± 2.1 and 27.9± 3.9 respectively in the control group. The concentration of SOD was significantly reduced in

the patients group compared to the control group (P=0.0001). The concentration of SOD was 55.1 6.9 in the patients and in the control the concentration was 64.5± 7.7 nmol/ml, table 1.

In the patients with spontaneous abortions, the positive serology for C. trachomatis either IgA and/ or IgG was 10%, figure 1.

In the study of oxidative stress markers in the patients with positive serology for C. trachomatis, there was significant increase in nitrite concentrations compared to patients negative for C. trachomatis serological markers (P=0.005). While reduced glutathione concentration had insignificant increase in patients positive for C. trachomatis with reduced SOD compared to patients with negative serology to C. trachomatis, P=0.6, P=0.07, respectively, table 2.

Table 1: Comparison of age and laboratory findings between patients and control.

Parameter	Patients with recurrent spontaneous abortions (n=100)	Control Females (n=100)	P
Age	29.9± 5.7	29.8± 5.9	P=0.9
C. trachomatis IgA	4	1	P=0.2
C. trachomatis IgG	8	2	P=0.005
SOD nmol/ml	55.1± 6.9	64.5± 7.7	P=0.0001
nitrite nmol/ml	27.9± 4.7	18.9± 2.1	P=0.0001
Reduced glutathione nmol/ml	31.4± 1.9	27.9± 3.9	P=0.0001

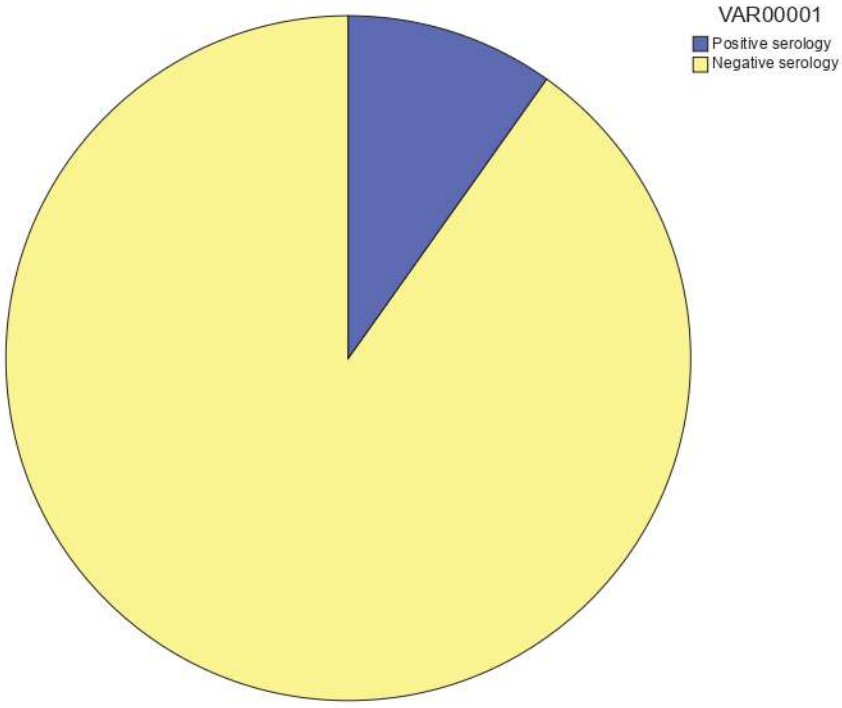

Figure (1): total seroprevalence to C. trachomatis among patients.

Table 2: Comparison between patients with positive antibodies IgA and/or IgG for C. trachomatis and patients with negative antibodies.

	Patients with positive IgG or/and IgA for C. trachomatis (n=79)	**Patients Negative for C. trachomatis IgG and IgA (n=21)**	**P**
Age	28.0± 5.1	30.1± 5.8	P=0.3
SODnmol/ml	53.7± 6.9	57.9± 6.4	P=0.07
nitritenmol/ml	31.6± 3.9	27.3± 4.6	P=0.005
glutathionenmol/ml	28.5 3.9	27.8± 3.9	P=0.6

Discussion

There are various etiologies of recurrent spontaneous abortions which may be attributed to alteration of the chromosomes, hormonal abnormalities, immunologic diseases and infections(17). The imbalance between oxidant/antioxidant may be implicated for such condition. The increase in the products of oxidative stress may lead to recurrent abortions via breaks in the double-strand DNA in the sperm and oocyte. (18, 19).

The concentration of oxidative stress products was significantly higher in the patients group compared to the control group (P=0.0001). The concentration of SOD was significantly reduced in the patients group compared to the control group (P=0.0001). Several studies have examined the role of OS in the incidence of pregnancy complications such intrauterine growth restriction or IUGR (20), preterm birth (21), preeclampsia (22, 23), and gestational diabetes (24, 25),**Similar results were reported with increase in different oxidative stress biomarkers and reduced in the antioxidant capacity in patients with repeated spontaneous abortions (13, 19).**

Among infections associated with female infertility is C. trachomatis. C. trachomatis infections are associated with wide varieties of pathological conditions such as salpingitis, pelvic inflammatory disease, ectopic pregnancyand femaleinfertility. C. trachomatis is a recognized agent of preterm labor and premature rupture of membranes (26, 27). Nevertheless, there are limited data about its association with miscarriage (26, 27).

In the present study,the prevalence of IgA and IgG for C. trachomatis in patients was 4% and 8% respectively and, in the control, subjects the prevalence of IgA and IgG were 1% and 2%. There was significant increase in seroprevalence of IgG for C. trachomatis in patients compared to control subjects. There were controversy in the association of C. trachomatis with spontaneous abortions as some studies reported no association between C. trachomatis active infection and spontaneous abortions (28, 29) while other studies reported significantly higher seroprevalence to C. trachomatis in patients with spontaneous abortions compared to females with normal pregnancy outcomes prevalence ranging between 11%-69% in miscarriages compared to 2-7% in healthy pregnant controls (30-32).

Persistant infection of C. trachomatis in female genital tract may lead to abortion via transfer to fetal tissue or endometrium. This effect may be associated with persistent infection and not during acute infection diagnosed by the presence of specific IgA (33).

The pathogenesis of C. trachomatis includes complex interaction between immune response and oxidative stress with the side effects of these reactions that lead to chronic endometritis, salpingitis, pelvic inflammatory disease and distal fallopian tube obstruction due to fibrosis (34, 35). In the present study there was significant increase in nitrite concentrations compared to patients negative for C. trachomatis serological markers (P=0.005). While reduced glutathione concentration had insignificant increase in patients positive for C. trachomatis with reduced SOD compared to patients with negative serology to C. trachomatis, P=0.6,

P=0.07, respectively. Previous studies reported the presence of imbalance in oxidant/antioxidant different groups of infertile in plasma, serum, follicular and peritoneal fluid of infertile women (36, 37). Also, a study reported the presence of this imbalance in patients with tubal infertility and spontaneous miscarriage associated with Chlamydia trachomatis infection (13).

The present study highlights that there was significant increase in oxidant stress biomarkers nitrite and reduced glutathione with significant reduction in superoxide dismutase in patients with repeated abortions. The prevalence of IgG to C. trachomatis was significantly prevalent in patients with recurrent abortion compared to control subjects. The nitrite was significantly correlated with positive serology to C. trachomatis.

References

1. M. Vander Borght and C. Wyns, "Fertility and infertility: definition and epidemiology," Clinical Biochemistry, vol. 62, pp. 2–10, 2018.
2. M. R. Sadeghil, "Introduction of sensitive and specific biomarkers can improve infertility treatment success rate," Journal of reproduction & infertility, vol. 13, no. 2, pp. 69-70, 2012.
3. A. A. Nardelli, T. Stafinski, T. Motan, K. Klein, and D. Menon, "Assisted reproductive technologies (ARTs): evaluation of evidence to support public policy development," Reproductive Health, vol. 11, no. 1, p. 76, 2014.
4. C. M. Combelles, S. Gupta, and A. Agarwal, "Could oxidative stress influence the in-vitro maturation of oocytes?" Reproductive Biomedicine Online, vol. 18, no. 6, pp. 864–880, 2009.
5. S. K. K. Jana, N. B. Chattopadhyay, R. Chakravarty, and B. K. Chaudhury, "Upper control limit of reactive oxygen species in follicular fluid beyond which viable embryo formation is not favorable," Reproductive Toxicology, vol. 29, no. 4, pp. 447–451, 2010.View at:
6. Pa Mardh. Tubal factor infertility, with special regard to chlamydial salpingitis. CurrOpin infect Dis 2004;17:49–52.
7. Tošić-Pajić J, Šeklić D, Radenković J, Marković S, Čukić J, Baskić D4, Popović S, Todorović M5, Sazdanović P.Augmented oxidative stress in infertile women with persistent chlamydial infection.Reprod Biol. 2017 Jun;17(2):120-125
8. Ray SD, Lam TS, Rotollo JA, Phadke S, Patel C, Dontabhaktuni A, et al. Oxidative stress is the master operator of drug and chemically-induced programmed and unprogrammed cell death: implications of natural antioxidants in vivo. Biofactor 2004;21:223–32.
9. Azenabor AA, Mahony JB. Generation of reactive oxygen species and formation and membrane lipid peroxides in cells infected with Chlamydia trachomatis. Int J Infect Dis 2000;4:46–50.
10. Rusconi B, Greub G. Chlamydiales and the innate immune response: friend or foe. FEMS Immunol Med Microbiol 2011;61:231–44.
11. Nsonwu-Anyanwu AC, Charles-Davies MA, Taiwo VO, Li B, Oni AA, Bello FA. Female reproductive hormones and biomarkers of oxidative stress in genital chlamydia infection in tubal factor infertility. J ReprodInfertil 2015;16:82–9.
12. de Carvalho LF, Abrao MS, Biscotti C, Sharma R, Agarwal A, Falcone T. Mapping histological levels of 8-hydroxy-2 dexyguanosine in female reproductive organs. J MolHistol 2013;44:111–6.
13. Tošić-Pajić J1, Šeklić D2, Radenković J2, Marković S2, Čukić J3, Baskić D4, Popović S5, Todorović M5, Sazdanović P6. Augmented oxidative stress in infertile women with persistent chlamydial infection.Reprod Biol. 2017 Jun;17(2):120-125.
14. Auclair C, Voisin E. Nitrobluetetrazolium reduction.Handbook of Methods for Oxygen Radical Research.Boka Ration: RA Greenwald; 1985.
15. Griess P. BemerkumgeenZu der Abhandlung per HH.Weselsky und BenediktUeBereinigeAzoverbindungenBerDtschChemges 1879;12:426–8.
16. Baker MA, Cerniglia GJ, Zaman A. Microtiter plate assay for the measurement of glutathione and glutathione disulfide in large numbers of biological samples. Anal Biochem 1990;190:360–5.
17. Christiansen OB, Steffensen R, Nielsen HS, Varming K. Multifactorial etiology of recurrent miscarriage and its scientific and clinical implications. GynecolObstet Invest 2008;66(4):257–67
18. Sumitha Prabhu PS, Aneesh P, Jiju JS, Reshma P, Dinesh Roy D. Evidence of increased

oxidative stress and DNA damages in women with recurrent abortions. Int J SciEng Res. 2015;6(5):15-19.

19. Ghneim HK, Alshebly MM. Biochemical Markers of Oxidative Stress in Saudi Women with Recurrent Miscarriage. J Korean Med Sci. 2016;31(1):98-105. doi:10.3346/jkms.2016.31.1.98
20. Holland O, Dekker Nitert M, Gallo LA, Vejzovic M, Fisher JJ, Perkins AV. Review: Placental mitochondrial function and structure in gestational disorders. Placenta. 2017;54:2- 9. doi:10.1016/j.placenta.2016.12.01
21. Mustafa MD, Pathak R, Ahmed T, et al. Association of glutathione S-transferase M1 and T1 gene polymorphisms and oxidative stress markers in preterm labor.ClinBiochem. 2010;43(13-14):1124-1128. doi:10.1016/j. clinbiochem.2010.06.018
22. Gupta S, Aziz N, Sekhon L, et al. Lipid peroxidation and antioxidant status in preeclampsia: a systematic review. ObstetGynecolSurv. 2009;64(11):750-759. doi:10.1097/ OGX .0b013e3181bea0ac
23. Wu F, Tian FJ, Lin Y, Xu WM. Oxidative Stress: Placenta Function and Dysfunction. Am J ReprodImmunol. 2016;76(4):258-271. doi:10.1111/aji.12454
24. Madazli R, Tuten A, Calay Z, Uzun H, Uludag S, Ocak V. The incidence of placental abnormalities, maternal and cord plasma malondialdehyde and vascular endothelial growth factor levels in women with gestational diabetes mellitus and nondiabetic controls. GynecolObstet Invest. 2008;65(4):227-232. doi:10.1159/000113045
25. Lappas M, Hiden U, Desoye G, Froehlich J, Hauguel-de Mouzon S, Jawerbaum A. The role of oxidative stress in the pathophysiology of gestational diabetes mellitus.Antioxid Redox Signal. 2011;15(12):3061-3100. doi:10.1089/ars.2010.3765
26. Baud D, Regan L, Greub G Emerging role of Chlamydia and Chlamydia-like organisms in adverse pregnancy outcomes. CurrOpin Infect Dis. 2008;21:70–6 10.1097/QCO.0b013e3282f3e6a5
27. Mårdh PA Influence of infection with Chlamydia trachomatis on pregnancy outcome, infant health and life-long sequelae in infected offspring. Best Pract Res ClinObstetGynaecol. 2002;16:847–64 10.1053/beog.2002.0329
28. Paukku M1, Tulppala M, Puolakkainen M, Anttila T, Paavonen J. Lack of association between serum antibodies to Chlamydia trachomatis and a history of recurrent pregnancy loss.FertilSteril. 1999 Sep;72(3):427-30.
29. Osser S, Persson K. Chlamydial antibodies in women who suffer miscarriage. Br J ObstetGynaecol. 1996;103:137–41.
30. Arsovic A, Nikolov A, Sazdanovic P, Popovic S, Baskic D. Prevalence and diagnostic significance of specific IgA and anti-heat shock protein 60 Chlamydia trachomatis antibodies in subfertile women. Eur J ClinMicrobiol Infect Dis 2014;33:761–766
31. Baud D, Goy G, Jaton K, Osterheld M-C, Blumer S, Borel N, Vial Y, Hohlfeld P, Pospischil A, Greub G. Role of Chlamydia trachomatis in miscarriage. Emerg Infect Dis 2011;17:1630–1635.
32. Wilkowska-Trojniel M, Zdrodowska-Stefanow B, Ostaszewska-Puchalska I, Redźko S, Przepieść J, Zdrodowski M. The influence of Chlamydia trachomatis infection on spontaneous abortions.Adv Med Sci 2009;54:86–90
33. Baud D, Regan L, Greub G. Emerging role of Chlamydia and Chlamydia-like organisms in adverse pregnancy outcomes. CurrOpin Infect Dis. 2008;21:70–6.
34. Nsonwu-Anyanwu AC, Charles-Davies MA, Taiwo VO, Li B, Oni AA, Bello FA.Female reproductive hormones and biomarkers of oxidative stress in genital chlamydia infection in tubal factor infertility. J ReprodInfertil 2015;16:82–9.
35. Chandra A, Surti N, ShKesavan, Agarwal A. Significance of oxidative stress in human reproduction. Arch Med Sci 2009;5:528–42.
36. Turan V, Sezer ED, Zeybek B, Sendag F. Infertility and the presence of insulin resistance are associated with increased oxidative stress in young, non-obese Turkish women with polycystic ovary syndrome. J PediatrAdolescGynecol 2015;28:119–23.
37. Soleimani Rad S, Abbasalizadeh S, GhorbaniHaghjo A, Sadagheyani M, Montaseri A, Soleimani Rad J. Evaluation of the melatonin and oxidative stress markers level in serum of fertile and infertile women. Iran J Reprod Med 2015;13:439–44.