Original Article

Detection Of Proapoptosis Bax- Protein In Aborted Women Infected With Toxoplasma Gondii In Wasit Province, Iraq

Shifaa Ali Abdulmohsin Al-Hachami [1], May Naji Al-Khanak [2], Jalal AbdulRazzaq Tofah Al-Azzawi [3]

[1, 2] Department of Biology, College of Science, University of Wasit, Wasit, Iraq
[3] Ministry of health, Wasit Directorate of health, Wasit, Iraq
Emails: shafaa9091@gmail.com [1], mayalkhanaq@uowasit.edu.iq [2], jalaltofah42@jmail.com [3]
* Corresponding author
DOI: 10.47750/pnr.2022.13.S08.138

Abstract

The current study aimed to determine the relationship between Toxoplasma gondii infection and the levels of proapoptosis Bax-protein in aborted women and normal delivery women, in Wasit province. The study included Two groups of women included patients group (300aborted women) and control group (50 non-infected normal delivery women), Serum samples from patients and control were collected and examined Anti- T. gondii (IgM, IgG and mixed infection IgM and IgG) antibodies by using cobas e411 technique then detected the levels of proapoptosis Bax- protein by using ELISA technique. The results show the percentage of Anti- T. gondii (IgM, IgG and mixed infection IgM and IgG) antibodies were 300/300 (11%,32% and 5.66%), respectively in patient, whereas 50/50no seropositive sera in control, there is high significant difference between patients and control women. The results also showed the total rate of proapoptosis Bax- protein was 146/300 (7.94±1.29) in T. gondii seropositive patients serum samples, whereas 50/50 (1.65± 0.57) in control serum samples by using ELISA, statistical analysis results indicated high significant differences between the prevalence of the infected patients and controlwomen.

Keywords: T. gondii, Abortion, Cobas e411, ELISA, Bax-protein

Introduction

Toxoplasma gondii has been defined as a very important obligate intra-cellular protozoan parasite in the human and veterinary animals, categorized in phylum Apicomplexan (Al-Hadraawy et al., 2019). The life cycle of T. gondii has two parts are the sexual cycle that occurs within cats and other felines which are the definitive hosts and the asexual cycle that occurs in the intermediate hosts – which are virtually any warm-blooded animal, including humans Also, T. gondii have three infectious stages are the sporulated oocysts that contain the sporozoites, the tachyzoites, and the tissue cysts that contain bradyzoites, all three stages are infectious to both the feline definitive hosts and the intermediate hosts (Shabo, 2015).

Humans can be infected through oocysts, or by the consumption of raw or undercooked meat containing tissue cysts. If a woman is infected for the first time during pregnancy, the parasite can be transmitted transplacentally, which may result in death of the fetus, or a baby born with nervous system abnormalities or chorioretinitis (Morris and Havelaar, 2021).

In recent years, serological and molecular diagnostic methods are frequently used for the diagnosis of T. gondii in

many countries, Serological screening for toxoplasmosis is performed in pregnant women in most of the countries during the first trimester (Ismael, 2021; Montoya and Liesenfeld, 2004). Assessing IgM and IgG in serum is normally used for detecting and differentiating between recent and latent T. gondii infections (Hampton, 2015).

Toxoplasmosis is one of the most common parasitic infections in humans, which is caused by Toxoplasma gondii, It is usually asymptomatic but primary infection in a pregnant woman can cause severe consequences in the fetus such as abortion (Kalantari et al., 2021). An important outcome of congenital toxoplasmosis is spontaneous abortion (Alvarado-Esquivel et al., 2014). Spontaneous abortion is the greatest common adverse pregnancy outcome From etiological perspective, It may perhaps happen as a result of genetic factors environmental, toxins infectious agents such as virus or bacteria and other maternal or paternal effects (chronic disease), (Zheng et al., 2017). Most of females are undergoing abortion or birth with congenital malformations due to one, two or all the following important causatives T. gondii, Rubella virus, and Cytomegalovirus (Majeed et al., 2020).

Apoptosis is an active form of cell death enabling individual cells to commit suicide that is necessary for the development and homeostasis of multicellular organisms (Zamaraev et al., 2017). It can be induced by many stimuli such as growth factor deprivation, exposure to ultraviolet rays, or exogenous factors. In addition, T. gondii can stimulate apoptosis during Toxoplasmic encephalitis, and initiate apoptosis in some cells and during specific stages of infection (Mammari et al., 2019).There are two major Apoptosis pathways: the death-receptor-induced extrinsic pathway and the mitochondria-apoptosome mediated apoptotic intrinsic pathway (Figure 1), (Li et al., 2015). The balance of these Bcl-2 family members determines whether the fate of cell is survival or death (Pithi, 2014).

Bax-protein is pro-apoptotic protein member of the Bcl-2 family, it localized at the mitochondrial membrane (Adams and Cory, 2018; Jurgensmeier et al., 2018). The pathway of bax-protein initiated by release of cytochrome c (is central in turning apoptosis and is regulated by the proapoptotic proteins and antiapoptotic proteins of the Bcl-2 family). The release of cytochrome c from mitochondria has been shown to promote the oligomerisation of a cytochrome c/APAF1/ procasepase 9 complexes (Figure 2) which leads to the induction of apoptosis (Hanash, 2014). The aimed of this study was to determine the prevalence of Toxoplasmosis in the serum of infected women compared with non-infected women by using e411COBAS test and detect the Proapoptosis Bax- protein by using ELISA test.

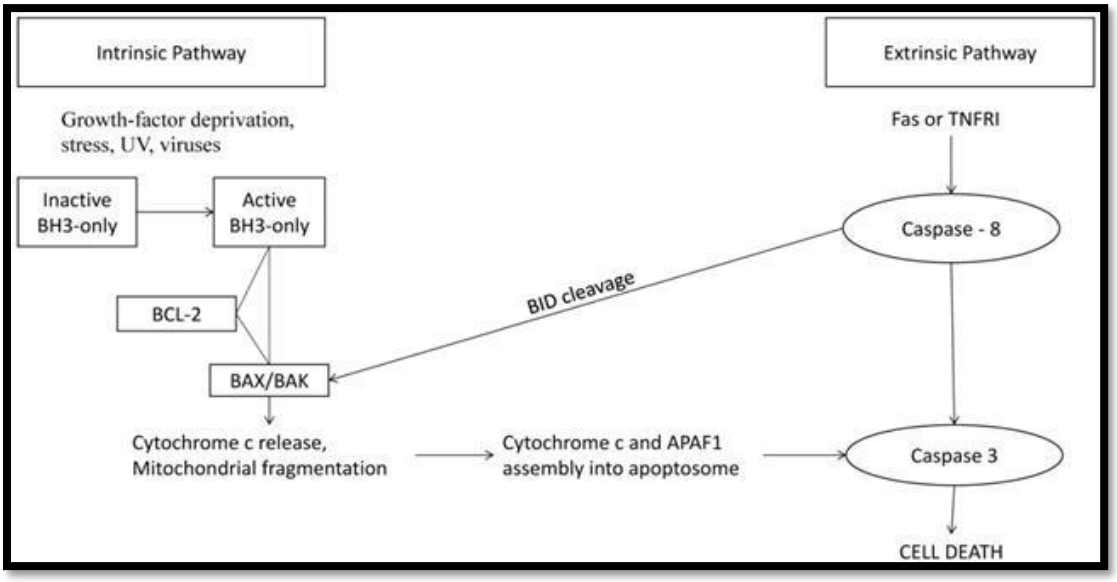

Figure (1): The intrinsic and extrinsic pathway of apoptosis (Chen et al., 2019)

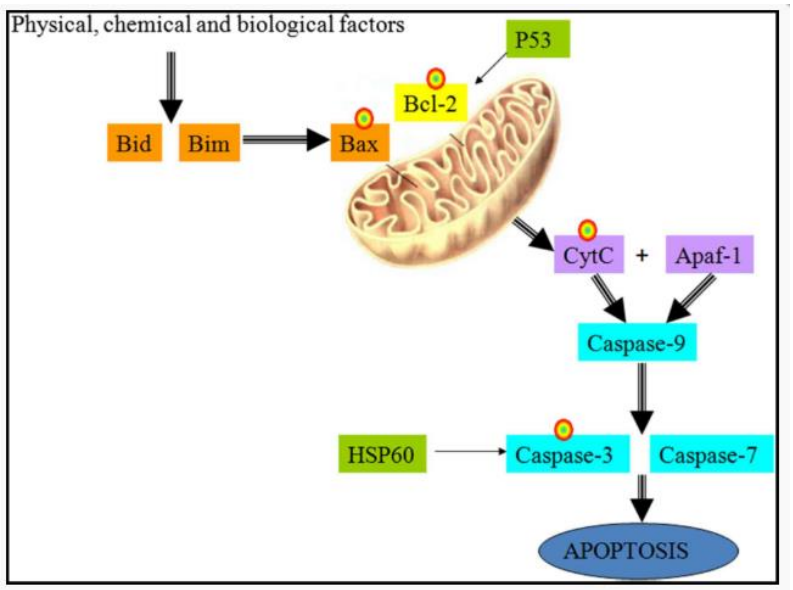

Figure (2): Mitochondrial signaling pathway (Zhang et al., 2017)

Materials and methods

Experimental Design

The study is carried out in collaboration Al-Zahra teaching hospital and Al-Kut hospital for Pediatrics and Gynae obstetrics. Total 350 serum samples are collected from January to November collected which include two different women's groups are used the Control (50 samples for normal pregnancy) and Patients (300 samples for women had spontaneous abortion.

Samples collection

Five ml of venous blood is collected from infected and control samples placed in a gel tube and put half an hour in a water bath at 37°C until blood is coagulated, After that centrifugation at 3500 rpm for 10 min to obtain a serum which is kept in the frozen at -20°C in sterile and clean plastic tubes until it is used for other serological and immunological tests (Hussein et al., 2022).

Detection of T. gondii using Cobas e411 test

Serum samples from 300 patients and 50 from control were tested for anti-T. gondii IgG, IgM and mixed infection IgG and IgM antibodies with the COBAS E411 and using Elecsys Toxo IgG Test Kit and Elecsys Toxo IgM Test Kit (Germmany company Roche®) ,which occurred by taking 10 µL of each samples pass through steps were: First incubation, Second incubation ,The reaction mixture and Results are determined automatically. Both assays for IgG and IgM were performed following the manufacturer's instructions (Hussein et al., 2022; Al-Salihy et al., 2018)

Detection the levels of proapoptosis Bax- protein

Toxo-seropositive Serum samples of patients and 50 control were tested for proapoptosis Bax- protein by using ELIZA technique, a test kits from the American company Elabscience ® was used, proapoptosis Bax- protein assay were performed following the manufacturer's instructions (Al-Qurabey,2018)

Statistical Analysis

The Data were analyzed by using SPSS computer software for findings numbers and percentages (%) and the T-test was used to compare between the percentages which ($P<0.05$) value was considered significant (Gharban and Yousif,

2020; Razooqi et al., 2022).

Results and Dissection

Detection of T. gondii by using Cobas e411 test

The results of current study observed that the total percentage of Toxoplasmosis infection were 146/300 (52%) ,which including anti- T. gondii IgM , IgG and mixed infection IgM and IgG antibodies seropositive samples infection in patients and control serum samples by using Cobas e411 were 33/300 (11%), 96/300 (32%) and 17/300 (5.6%) ,respectively, in patients serum samples, whereas no sera positive from 50 tested control serum samples, statistical analysis results indicated high significant differences between the prevalence of the infected patients and control as shown in table (1)

Table 1: Prevalence Rate of T. gondii in Aborted Women and Control According to IgM, IgG by Using Cobas e411 Test

Antibodies	Abortedwomen (Total No.=300)		Control(Total No.=50)	
	Toxo. Positive		Toxo. Positive	
	No.	%	No.	%
IgM	33	11	0	0
IgG	96	32	0	0
IgG and IgM	17	5.66	0	0
Total	146	52	0	0

Significant difference at a level ($p<0.05$)

The present results agreement with the results by Al-Salihy et al. (2018) in Diyala City recorded the percentage of seropositive for Anti T. gondii IgM was 2/100 (2%) whereas, the percentage of seropositive for Anti T. gondii IgG was 19/100 (19%) using Cobas e411 test. Also Al-Taei and Jasim (2021) in Tikrit Province recorded the seropostive for IgM anti-Toxoplasma and IgG anti-Toxoplasma were 75/100 (3.6 ± 0.3), 75/100 (1.037 ± 0.107), respectively in aborted women, whereas 25/100 (0.348 ± 0.065), (1.037 ± 0.107), respectively in control group, there was a higher significant difference using Cobas e411 test.

Also, The present results agreement with the results by Parlaket al.,(2015) in Van/ Turkey , recorded the rates of anti-Toxoplasma, IgM antibody positivity were 99/9156 (1.1%) whereas IgG antibody positivity was 172/ 457 (37.6%) in the serum samples of pregnant women receiving prenatal care , there was a higher significant difference, using Cobas e411. The present results agreement with the results by Shabo (2015) in Geizera State/ Sudan which recorded that 2/96 (2.1%) were seropostive for IgM anti-Toxoplasma antibodies, while 40/96 (41.6%) were seropositive for IgG anti-Toxoplasma antibodies in women with spontaneous abortions by using Cobas e411.

In contrast, these results disagree with a previous study done in Thi-Qar by Hadi (2011) who showed that anti- T. gondii IgM , IgG and mixed infection IgM and IgG antibodies were 16/40 (40%),4/40(11%),and 4/18(24%) respectively in aborted women by using miniVIDAS technique. The present results disagreement with the results by Hadi et al. (2016), in Diwanyia, recorded the rates of anti- T. gondii IgM , IgG and mixed infection IgM and IgG antibodies were 55/125 (4%) , 5/125 (44%),and 0/125(0%), respectively in aborted women ,whereas the healthy women were 0/30(0%) for all antibodies by using ELISA technique .

The results showed high significant differences between aborted and healthy women at ($p<0.05$),This explains the effect of T. gondii parasite on the pregnant women, The high rates of infection by Toxoplasmosis in women in this study is mainly may be acquired by ingestion of undercooked or raw meat containing viable tissue cyst or by ingestion of food or water that contaminated with oocyst shed by cats such as similar study (Liu et al., 2015).

The results showed high significant differences between total percentage of IgM and IgG antibodies, because most IgG-positive toxoplasmosis cases were sufficient to determine the presence of chronic infection; whereas IgM-positive toxoplasmosis cases contributes to the presence of acute infections. Serological tests were useful for T. gondii diagnosis by detecting antibodies in serum and determining the phase of infection in the acute and chronic phases (Jassam, 2020).

2. Detection the levels of proapoptosis Bax- protein

The results of current study observed that the total rate of Bax- protein in T.gondii seropositive infected aborted women and control serum samples by using ELISA were 146/300 (7.94 ± 1.29) in T. gondii seropositive infected aborted women, whereas 50/50(1.65± 0.57) in control serum samples, statistical analysis results indicated high significant differences between the prevalence of the infected aborted women and control groupas shown in table(2)

Table (2): Proapoptosis Bax- protein Levels in T. gondii seropositive Aborted Women and control group

Group	Mean ± SD (Pg/ml)	S.E
Toxo-seropositive Aborted Women	**7.94±1.29**	**1.15**
Control	**1.65± 0.57**	**0.12**
P-value¥	**0.001****	

The results of current study agreement with Elsalam et al., (2021) recorded that the expression of Bax proteins was 66/108 (35.3%) in T. gondii–positive women with recurrent abortion whereas, 20/108 (12.3%) in women with no history of abortion as a control group by using Quantitative polymerase chain reaction (qPCR).

The results of current study disagreement with Lv et al. (2016) recorded that the expression of Bax-mRNA in the spontaneous abortion women group were 40/40 (0.15±0.06), whereas, 30/30 (0.46±0.08) in the healthy womencontrol group by using Quantitative real-time PCR.

Differences in results among these studies may be attributed to timing of samples collection after abortion and to immune competent of volunteers subjects as well as to size of data included in the study (Aldabagh et al., 2018). Proapoptosis Bax- protein, which are located in the mitochondria, play an important role in apoptotic pathways, the different constitutive expressions of Bax are related to the Bcl2 / Bax ratio, leading to the failure of mitochondrial disruption associated with conformational changes, The Bax gene has an important role in pregnancy loss and the variations of this gene could help in the assessment of recurrent pregnancy loss (Seyedhassani et al., 2011).

On the other hand T. gondii inhibits the mitochondrial apoptotic pathway via interference with mitochondrial translocation and/or activation of the effector molecules Bax and Bak. This is achieved without altering the levels of pro-apoptotic regulators of the host, thus suggesting direct parasite interference with Bax. Also, triggering of the intrinsic pathway of apoptosis does not induce cell death of parasite-infected cells, thus increasing the viability of host cells (Hippeet al., 2009).

Conclusions

Toxoplasma gondii was the most widespread species in women and therefore the current study detect that the prevalence of Toxoplasmosis in aborted women by using e411 COBAS test. In this study observed an increase the proapoptosis Bax- protein in the serum of infected women compared with control group, this indicating their role in the immune response against infection of this parasite. It is necessary to establish efficient strategies to control and

reduce T. gondii transmission in women in Wasit province.

References

1. Adams, J. M., and Cory, S. (2018). The BCL-2 arbiters of apoptosis and their growing role as cancer targets. Cell Death and Differentiation, 25(1):27-36.
2. Aldabagh, M.,A.,H., Hachim, S.K., Qassim,K.W., Al-Mayah, Q.S., Hassan, J.S. and Salloom, D.F.(2018). Immune Profile in Aborted Iraqi Women with Toxoplasmosis. Medical Journal of Babylon , 15 (1) :48-52.
3. Al-Hadraawy,S.K., Alzeyadi, M., Shakir,A.,and Al-dujaili,A. (2019). Detection of Toxoplasma gondii genotypes in abortion women by RFLP-PCR in Al-Najaf Al-Ashraf province. IOP Conf. Series: JPhy, 5: 1-12.
4. Al-Qurabey,H.E.S.(2018). phagocytic activity and cytokines level in pregnant woman infected with Toxoplasma gondii.M.Sc. Thesis, Waist University, Iraq.
5. Al-Salihy, S.R. ,Hussain,H.A., Darweesh,N.H. and Wadi,H.H.(2018).THE EFFECT OF TOXOPLASMA GONDII SEROPOSITIVITY ON SIGNSANDSYMPTOMSOFMENOPAUSEINIRAQIWOMEN.DiyalaJournalofAgriculturalSciences,10(Specialissue):348-357.
6. Al-Taei,Z.K.M. and Jasim,M.M.(2021). Study of the relationship between toxoplasmosis and its effect on the level of the hormones prolactin and progesterone and the level of interleukin 27 among aborted women in the city of Tikrit Microbial Science Archives , 1 (1): 18-22.
7. Alvarado-Esquivel, C., Pacheco-Vega, S.j., Hernandez-Tinoco,j., CentenoTinoco,M.M., I. Beristain-Garcia,I., Sanchez-Anguiano, L.F.,O. Liesenfeld, E. RabagoSanchez,E.andBerumen-Segovia,L.O .(2014).Miscarriage history and Toxoplasma gondii infection: a cross-sectional study in women in Durango City, Mexico Eur. J. Microbiol. Immunol.4 : 117–122.
8. Elsalam, SA; Mansor, AE; Sarhan, MH; Shalaby, AM; Gobran, MA; Alabiad, MA.(2021). Evaluation of Apoptosis, Proliferation, and Adhesion Molecule Expression in Trophoblastic Tissue of Women With Recurrent Spontaneous Abortion and Infected With Toxoplasma gondii. International Journal of Gynecological Pathology, 40 (2): 124-133.
9. Gharban, H. A., and Yousif, A. A. (2020). Serological and Molecular Phylogenetic Detection of Coxiella burnetii in Lactating Cows, Iraq. The Iraqi Journal of Veterinary Medicine, 44(E0), 42-50.
10. Hadi, H. S., Kadhim, R. A. and Al-Mammori, R. T. O. (2016).Seroepidemiological aspects for Toxoplasma gondiiinfection in women of Qadisiyah province, Iraq.International Journal of PharmTech Research, 9(11): 252–259.
11. Hampton,M.M. (2015) Congenital toxoplasmosis: a review, Neonat. Netw.34 :274–278.
12. Hanash,S.H.(2014). P53 and Bcl-2 Genes Expression in Bladder Cancer Patients in Wasit province /Iraq. M. Sc. Thesis, College of Science, Wasit of University.
13. Hippe, D., Weber, A., Zhou, L., Chang, D. C., Häcker, G., andLüder, C. G. (2009). Toxoplasma gondii infection confers resistance against BimS-induced apoptosis by preventing the activation and mitochondrial targeting of pro-apoptotic Bax. Journal of cell science, 122(19):3511-3521.
14. Hussein, T.W., Mohammed,K.I.A. and Mohammed,A. (2022). Levels of Interleukin-6,17,Complement and Dopamine in Infected Women with Toxoplasmosis. South Asian Res J Pharm Sci, 4(3): 54-59
15. Ismael,S.S.(2021).Diagnostic methods and protocols used in investigating Toxoplasma gondii in humans: A review Baghdad Journal of Biochemistry and Applied Biological Sciences ;2(04):181-186.
16. Jassam,H.M.S.(2020). The Factors effects in Abortion in Women with Toxoplasmosis in Al-Anbar Governorate.M.Sc. Thesis, College of Science, University of Anbar ,Iraq.
17. Kalantari, N., Gorgani-Firouzjaee,T., Moulana,Z., Chehrazi,M. and Ghaffari,S.(2021). Toxoplasma gondii infection and spontaneous abortion: A systematic review and meta-analysis.Microbial Pathogenesis ,158:105070.
18. Li, X., Zhao, Y., Chen, B., Zhu, Z., Kang, Q., Husain, T. and Zhang, B. (2022). Inhalation and ingestion of synthetic musks in pregnant women: In silico spontaneous abortion risk evaluation and control. Environment International, 158, 106911.
19. Liu, Q., Wang, Z. D., Huang, S. Y., and Zhu, X. Q. (2015).Diagnosis of toxoplasmosis and typing of Toxoplasma gondii. Parasites and vectors, 8(1): 1-14.
20. Lv, X., Cai, Z. and Li, S. (2016). Increased apoptosis rate of human decidual cells and cytotrophoblasts in patients with recurrent spontaneous abortion as a result of abnormal expression of CDKN1A and Bax. Experimental and Therapeutic Medicine, 12(5): 2865-2868
21. Majeed,H. T. , Hasan T. H. and Aljanaby, A.A.J.(2020). Epidemiological study in women infected with Toxoplasma gondii, Rubella virus, and Cytomegalo virus in Al-Najaf GovernorateIraq. International Journal of Pharmaceutical Research, (1): 1442-1447.
22. Mammari, N., Halabi, M. A., Yaacoub, S., Chlala, H., Dardé, M. L., and Courtioux, B. (2019). Toxoplasma gondiiModulates the Host Cell Responses: An Overview of Apoptosis Pathways. Biomed Research International, 6152489-6152489.:1-11.
23. Montoya, J. G. and Liesenfeld, O. (2004).Toxoplasmosis. Lancet, 363:1965-1976.
24. Morris, J. G. and Havelaar, A .(2021). Foodborne Infections and Intoxications (Fifth Edition) ,Chapter 20 - Toxoplasma gondii, 347-361.
25. Parlak, M., Çim, N., NalçaErdin, B., Güven, A., Bayram, Y., andYıldızhan, R. (2015).Seroprevalence of Toxoplasma, Rubella, and Cytomegalovirus among pregnant women in Van. Turkish journal of obstetrics and gynecology, 12(2): 79–82.
26. Pithi,C. (2014). Molecular mechanisms of chemotherapeutic resistance in lung cancer. Thai Journal of Pharmaceutical Sciences, 38:1-4.

27. Razooqi, M. A., Gharban, H. A., and Al-Kaabi, M. A. (2022). Molecular and Seroprevalence of Toxoplasmosis in Goats' Blood and Milk in Iraq. Archives of Razi Institute, 77(5), 1749-1755
28. Seyedhassani,S.M., Houshmand,M., Kalantar,S.M., Aflatoonian,A., Modabber,G., Hashemi-Gorji,F., Hadipour,Z.(2011).BAX pro-apoptotic gene alterations in repeated pregnancy loss.Arch Med Sci. ,7(1):117–122.
29. Shabo,N.E.I. (2015). Screening of Toxoplasmosis Antibodies in Women with Spontaneous Abortion Attending Wad MedaniObstetrics and Gynecology Teaching Hospital, Geizera State, Sudan. M. Sc. Thesis, University of Gezira , Sudan.
30. Ursu, R. G., Costin, D., Iancu, L. S., and Nemescu, D. (2015). The clinical utility of TORCH testing of pregnant women from northeastern Romanian population: application of electrochemiluminiscence assay. In 2015 E-Health and Bioengineering Conference (EHB):1-4.doi: 10.1109/EHB.2015.7391504.
31. Zamaraev, A. V., Kopeina, G. S., Prokhorova, E. A., Zhivotovsky, B., and Lavrik, I. N. (2017). Post-translational modification of caspases: the other side of apoptosis regulation. Trends in cell biology, 27(5): 322-339.
32. Zheng, D., Li, C., Wu, T. and Tang, K. (2017). Factors associated with spontaneous abortion: a cross-sectional study of Chinese populations. Reproductive health, 14(1): 1-9.