ISSN: 1813-1638

The Medical Journal of Tikrit University

Available online at: www.mjotu.com

Evaluation the relation of TNF-alpha in aborted women who suffer from bacterial vaginitis

Nadya I. Salih [*(1)], **Maisaloon F. Hassan** [(2)]

(1) Department of Microbiology
College of Medicine
Tikrit University
Salahaldeen
Iraq
(1) Kirkuk Health Directorate
Kirkuk
Iraq

***Keywords*:**

TNF-α
Abortion
Kirkuk.
Bacterial vaginitis

A R T I C L E I N F O

Article history:

Received 03 July 2018
Accepted 05 Nov 2018
Available online 01 Dec 2018

ABSTRACT:

Background The role of infectious diseases in recurrent miscarriage is not clarified yet, but proposed an incidence of 0.5–5%. There are some candidate infectious diseases such as Listeria monocytogenes, Toxoplasma gondii, rubella, herpes simplex virus (HSV), measles, cytomegalovirus, and coxsackie viruses.

Aim: This study was aimed at evaluation of the relation of TNF-α level in aborted women infected with bacterial vaginitis.

Material and Method A cross sectional study was carried out in Kirkuk city from the beginning of February 2018 to the end of May 2018, deep vaginal swab was collected from 100 women with recurrent abortion and 50 pregnant women (as control group) that belonged to different geographical areas in Kirkuk city and admitted to Kirkuk General Hospital. The swabs samples were cultured on blood agar and MacConkey agar for 24 hour and secondary cultures were done for resulted culture for isolate specific bacteria. Five ml of blood was collected from each women enrolled in this study for TNF-α test using ELISA technique.

Results The study showed that 57% of women with recurrent abortion have positive high vaginal swab (HVS) culture comparing with 48% of pregnant women (control group). The current study showed that 86% of women had aborted in the 1st trimester of pregnancy and the least rate of abortion was in the 3rd trimester. The study showed that 52.25% of aborted women were belonged to the age group 27-36 years and highest rate of isolated bacteria from the HVS culture of aborted women was S. aureus (45.61%) while Proteus spp. was not isolated from aborted women. The study found that the highest mean of TNF-α level was denoted among women with abortion comparing with the control group (26.79±9.4 v.s 9.236±0.6 pg/ml) with highly significant relation of TNF-α level with abortion. The study showed that the highest TNF-α mean levels was recorded among women who aborted in the 1st trimester (28.75±8.76 pg/ml) and the lowest mean was noted among the 3rd trimester aborted women with highly significant relation among the groups.

Conclusions It was concluded that the there was a highly significant relation of TNF-α level with abortion specially with women suffered from bacterial vaginosis.

DOI: http://dx.doi.org/10.25130/mjotu.24.02.01

**Corresponding author E mail:* micro999bio@tu.edu.iq

Introduction

Recurrent miscarriage (RM) is defined as the occurrence of three or more consecutive losses of pregnancy. According to this definition, it affects about 1% of couples trying to have a baby [1]. However, many clinicians define RM as two or more losses; this increases the percentage of RM from 1% to 5% of all couples trying to conceive [2]. The role of infectious diseases in recurrent miscarriage is not clarified yet, but proposed an incidence of 0.5–5% [3]. There are some candidate infectious diseases such as *Listeria monocytogenes*, *Toxoplasma gondii*, rubella, herpes simplex virus (HSV), measles, cytomegalovirus, and coxsackie viruses. Infectious diseases may cause pregnancy loss by the following mechanisms such as direct infection of the uterus, fetus, or placenta, placental insufficiency, chronic endometritis, endocervicitis, amnionitis, or intrauterine miscellaneous infections[4]. Immunological rejection of the fetus due to recognition of paternal antigens by the maternal immune system, resulting in abnormal immune cells and cytokine production, is postulated to be one cause of unexplained pregnancy loss [4]. Cytokines have traditionally been divided into families dependent upon the immune cell of origin and the immunological effects that they bring about[5]. Several mechanisms were proposed for the pro-abortogenic effects of TNF-α, including trophoblast invasion and placentation and induction of the expression of pro-apoptotic genes in human fetal membranes, which in turn accelerates membrane degradation and thus increases the susceptibility to premature rupture. TNF-α was also described to facilitate miscarriage indirectly by activating NK cells or macrophages [6,7]. This study was aimed at evaluation of the relation of TNF-α level in aborted women infected with bacterial vaginosis.

Material and methods

A cross sectional study was carried out in Kirkuk city from beginning of February 2018 to the end of May 2018, deep vaginal swab was collected from 100 women with recurrent abortion and 50 pregnant women (as control group) that belonged to different geographical areas in Kirkuk city were admitted to Kirkuk General Hospital. The swabs samples were cultured on blood agar and MacConkey agar for 24 hour and secondary cultures were done for resulted culture for isolate specific bacteria. Five ml of blood was collected from each women enrolled in this study, the obtained sera were aspirated and transferred to Eppendorf tubes and stored in deep freeze at -20°C for late TNF-α test using ELISA technique.

Statistical Analysis :

Computerized statistically analysis was performed using IBM SPSS V23.0.0 statistic program. Comparison was carried out using; Chi square and T-Test.

Results:

The study showed that 57% of women with recurrent abortion have positive HVS culture comparing with 48% of pregnant women (control group), Table 1. The current study showed that 86% of women had aborted in the 1st trimester of pregnancy and the least rate of abortion was in the 3rd trimester, Figure 1. The current study showed that highest rate of isolated bacteria from the HVS culture of aborted women was *S. aureus* (45.61%) and the higher rates of isolated bacteria from pregnant women were 29.17% for each of *E coli* and *K. pneumonia* and while *Proteus* spp. was not isolated from aborted women, Table 2. The study found that the highest mean of TNF-α level was denoted among women with abortion comparing with the control group (26.79±9.4 *v.s* 9.236±0.6 pg/ml) with highly significant relation of TNF-α level with abortion, Table 3. The study showed that the highest

TNF-α mean levels was recorded among women who aborted in the 1st trimester (28.75±8.76 pg/ml) and the lowest mean was noted among the 3rd trimester aborted women with highly significant relation among the groups, Table 4.

Table 1: Distribution of HVS culture in recurrent aborted women and the control group.

Results of vaginal swab culture	Study groups			
	Recurrent abortion women		Pregnant women (control)	
	No.	%	No.	%
Pathogenic bacteria	57	57	24	48
Normal flora	43	43	26	52
Total	100	100	50	100
X2 = 1.08	***P. value* = 0.29**		**Non Significant (NS)**	

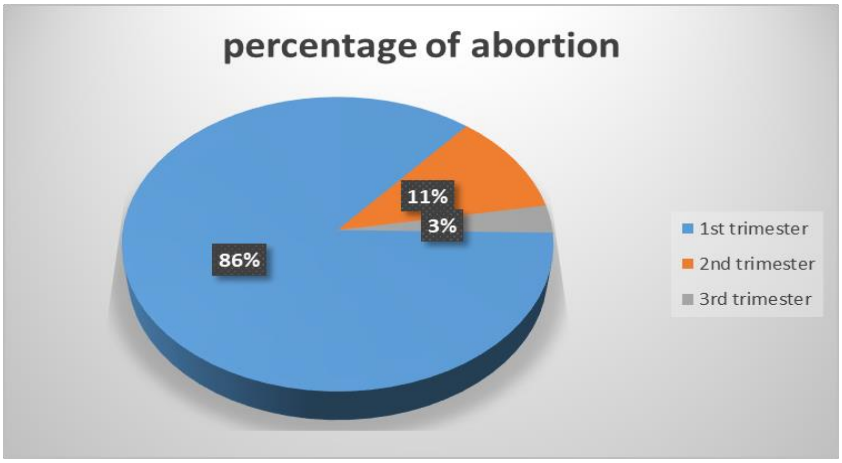

Figure 1: Distribution of aborted women according to trimester of pregnancy.

Table 2: Distribution of isolated bacteria among study groups.

Isolated Bacteria	Positive HVS cases			
	Aborted women		Pregnant women (control)	
	No.	%	No	%
Escherichia coli	17	29.82	7	29.17
Staphylococcus aureus	26	45.61	4	16.67
Klebsiella pneumoniae	14	24.57	7	29.17
Proteus	0	0	6	25.0
Total	57	100	24	100
X2= 21.03	P. value=0.0003		HS	

Table 3: Estimation of TNF-α level in aborted women and the control group.

TNF-α level (pg/ml)	Study groups		T. test	*P. value*
	Recurrent abortion women	Pregnant women (control)		
No.	100	50	13.04	0.0001 HS
Mean	26.79	9.236		
SD	9.4	0.6		

Table 4: Distribution of TNF-α mean levels according to trimester of abortion.

TNF-α level (pg/ml)	Gestational time of abortion			F. ratio	*P. value*
	1st trimester	2nd trimester	3rd trimester		
No.	86	11	3	17.42	0.00001 Highly Significant
Mean	28.75	15.22	13.3		
SD	8.76	0.62	0.4		

Discussion:

In present study, the overall prevalence of vaginal infections (57%) was coherent with reports in Kirkuk (33.2 %) [7], Iran (27.6 %) [8] India (34.7 %) [9], in Vietnam (49.5 %) [10], and Pakistan (44 %) [11]. This variation might be methodology difference in isolation and identification of etiologies of vaginal infections. Moreover, environmental factors and difference on the actual study participants might also explain the above discrepancy. The most prevalent bacteria were *S. aureus* (45.61%) being also found by Manges *et al* [12]. Hayat *et al* [13] and Mumtaz *et al* [14] found that causative organisms of were *E. coli* in less than one-third of cases followed by *Klebsilla* less than one quarter and more than 5% were Proteus. *Staphylococcus aureus* may just be organisms causing local vaginal infection as they did not occur in the endocervix and may not have been responsible for the ascending upper genital tract infection in septic abortions. However, *Staphylococcus*

aureus is one of the several organisms that have been reported to cause septic abortion [15]. Advancing maternal age and a patient history of a previous spontaneous abortion are the two leading factors associated with a greater risk of spontaneous abortion [16]. Sundari *et al* [18] reported in their study that the majority of the sample was reported at first trimester of pregnancy. Hassan *et al* [19] and Israa [20] who reported a similar results. Ra'ad *et al* [21] in a study of vaginitis in married women in Tikrit city found that women with 1st trimester of abortion recorded the highest rate of abortion. Several studies have reevaluated the connection between abortion risk and TNF-α polymorphisms [5,6]. In addition, results of this study are harmonized with previous report that Th-1 cytokines levels (IFN-γ, TNF-α) are augmented during abortion and may be accused for abortion, as Th-1 cytokines, IFN-γ inhibited in vitro proliferation of human trophoblast cells [22]. Abdullah *et al* [23] revealed a significant association between raised serum TNF-α and first trimester pregnancy loss, strengthen the relation between these agents and fetal rejection and age does not influence the levels of TNF-α in studied women sera with first trimester pregnancy failure. In addition, ageing is associated with increased inflammatory activity in the blood that elderly cohort had increased circulating levels of TNF- α [24]. Infection and inflammation have both ability to change the levels of circulating cytokine levels [22]. Al-Hilli [25] in here study compared patients with recurrent miscarriage with normal pregnant women in the first trimester and found a highly significant difference in maternal serum TNF- α level between the two groups in recurrent miscarriage and control group respectively. Similar results were obtained in a study done by Daher *et al* [26] who measure the level of a number of cytokines (TNF-α, interleukin-6 & others) in women with recurrent pregnancy loss and

compare them with control group, they found that TNF α was significantly higher in women with recurrent pregnancy loss compared to control. Another study done by Kim *et al* [27] who investigated immunophenotypic characteristics of peripheral blood mononuclear cells & evaluate Th1 cytokine (TNF-α) production in women with recurrent spontaneous abortions, they observed a significantly higher level of TNF- α in recurrent spontaneous abortion when compared to controls.

Conclusions:

It was concluded that the there was a highly significant relation of TNF-α level with abortion specially with women suffered from bacterial vaginosis .

References.

1- Quaas AM. Infertility and Recurrent Pregnancy Loss. Glass' Office Gynecology. 2014:205-233.

2- Işik G, Demirezen Ş, Dönmez HG, Beksaç MS. Bacterial vaginosis in association with spontaneous abortion and recurrent pregnancy losses. I Cytol /Ind Acad Cytologists 2016 ;33(3):135.

3- Cicinelli E, Matteo M, Tinelli R, *et al*. Chronic endometritis due to common bacteria is prevalent in women with recurrent miscarriage as confirmed by improved pregnancy outcome after antibiotic treatment. Reprod Sci 2014;21(5):640-647.

4- Basu J, Agamasu E, Bendek B, Salafia C, Mishra A, Benfield N, Prasad P, Mikhail M. 736: Placental tumor necrosis factor-α levels throughout gestation in normal pregnancy. Am J Obstet Gynecol 2015;212(1):S359-60.

5- Basu J, Agamasu E, Bendek B, Salafia C, Mishra A, Benfield N, Prasad P, Mikhail M. 736: Placental tumor necrosis factor-α levels throughout gestation in normal pregnancy. Am J Obstet Gynecol 2015;212(1):S359-60.

6- Li S, Wang L, Xing Z, Huang Y, Miao Z. Expression level of TNF-α in decidual tissue and peripheral blood of patients with recurrent

spontaneous abortion. Central-Euro J Immunol 2017;42(2):156.

7- Kadir MA, Sulymaz MA, Dawood IS, Shams- Eldin S. *Trichomonas vaginalis* and associated microorganisms in women with vaginal discharge in Kerkuk-Iraq. Ankara Med J. 2014;14(3):91–99.

8- Bahram A, Hamid B, Zohre T. Prevalence of bacterial vaginosis and impact of genital hygiene practices in non-pregnant women in Zanjan, Iran. Oman Med J. 2009;24:288–293.

9- Gupta G, Nandwam S, Agarwal A. Prevalence of candidiasis, trichomoniasis and bacterial vaginosis among women of reproductive age group. Indian J Pub Heal Res Dev. 2013;4(2):94–98.

10- Go VF, Quan VM, Celentano DD, Moulton LH, Zenilman JM. Prevalence and risk factors for reproductive tract infections among women in rural vietnam. Southeast Asian J Trop Med Pub Heal 2006;37:185–189.

11- Mathew R, Sudhakshina R, Kalyani M, Jayakumars S, Lai B, Banu S. Microbiological profile of vaginosis among women of the reprioductive age group, who attended a tertiary care Hospital. JCDR. 2011;8(5):1548-15451.

12- Manges AR, Johnson JR, Foxman B, O'bryan TT, Fullerton KE, Riley LW. Widespread distribution of urinary tract infections caused by a multidrug-resistant Escherichia coli clonal group. New England JMed 2001;345(14):1007-1013.

13- Hayat IM, Nagat SS, Nermine N, Zeinab AB. Prevalence of Vaginal Infection and Associated Risk Health Behaviors Among Married Women in Ismailia City. Int. J. Curr Microbiol App Sci 2015;4(5):555-567.

14- Mumtaz S, Ahmad M, Aftab I, Akhtar N, ul Hassan M, Hamid A. Aerobic vaginal pathogens and their sensitivity pattern. J Ayub Med Coll Abbottabad. 2008;20(1):113-117.

15- Grudzinkas JG. Miscarriages, ectopic pregnancy and trophpoblastic disease. In Edmonds K. (Ed) Dewhurst Textbook of Obstetrics and Gynaecology for Post th Graduate students. 7th edition. London. Blackwell Science. 2012. 61-75.

16- Sundari MT. Sundari TK. Can health education improve pregnancy outcome?: Report of a

grassroots action-education campaign. J Family Welfare 1993;39 (1): 1-12.

17- Hassan MA, Killick SR. Is previous aberrant reproductive outcome predictive of subsequently reduced fecundity?. Human reproduct 2005;20(3):657-664.

18- Ali IH. Effect of toxoplasmosis as a cause of abortion in pregnant women. Tikrit J Pure Sc 2009;14(2):202-208.

19- Ra'ad AZ, Ahmed TH, Najim WS. Diagnosis of vaginitis in married women by microbiogical and molecular methods in Tikrit City. Tikrit Med J 2015;20(1).

20- Paradisi R, Porcu E. Maternal Serum Levels of Pro- Inflammatory Cytokines in Missed and Threatened Abortion. Mat J Reproductive Immunol 2003;50(4):301 –308.

21- Abdullah GA, Mahdi NK. The Role of Cytokines among Women with Spontaneous Miscarriage. Med J Islamic World Acad of Sc 2013;21(3):119-124.

22- Bruunsgaard H, Skinhøj P, Pedersen AN, Schroll M, Pedersen BK. Ageing, tumour necrosis factor- alpha (TNF- α) and atherosclerosis. Clin Exp Immunol 2000;121(2):255-260.

23- Al-Hilli NM. Maternal Serum Tumor Necrosis Factor-alpha in Patients with Missed and Recurrent Miscarriage Med J Babylon 2009;6 (3-4): 521-526.

24- Daher S, Camano L, Cytokines in recurrent pregnancy loss. J Reprod mmunol 2004; 62(1-2): 151-157.

25- Kim HR, Park AJ, Lee MK. CD56/CD16 expression on mononuclear cells and concentration of serum TNF-alpha in recurrent spontaneous abortion. Korean J Lab Med 2006; 26(3): 198-203.