Molecular Detection of Tumor Necrosis Factor-Alpha (TNF- a) Gene in Mycotic aborted Placenta of Ewes Using RT-PCR

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Abstract

Background: Abortion is associated with altered inflammatory molecule expression at the maternal-fetal interface. (TNF-alpha) are inflammatory proteins and display cytokine as a function that enhances the leukocyte recruitment to the inflammatory site.

Objectives: This work aimed at establishing the TNF-alpha gene expression in mycotic aborted placental animals as opposed to normal delivery.

Materials and Method: Using the technique Real Time-Polymerase Chain Reaction (RT-PCR), TNF-α DNA, tested in the mycotic aborted Placenta group (15 cases), normal delivery group (15 cases).

Results: The levels of TNF-α DNA expressed in the Placenta varied significantly between the two groups ($P<0.05$), respectively. The expression of TNF during abortion was significantly higher than in normally delivered models.

Conclusion: These findings indicate that both cytokines Th1 and Th2 play a key role in the pathogenesis of abortion. TNF alpha is likely a source of abortion's genetic susceptibility. There had been a strong correlation between the two cytokines and abortion.

Keywords: *Tumor necrosis,Factor-alpha, (TNF- a) gene, RT-PCR, Mycotic abortion.*

Introduction

Inflammatory processes caused by host resistance to infection or by infection-independent immune disorders pose a major challenge for successful pregnancy[1,2]. Inflammatory mediators, particularly tumor necrosis factor-a (TNFA), which act on the gestational tissues to damage the supply and function of the placental blood[3] and cause fetal injury[4], eventually leading to placental and fetal death. In fact, the predominance of cellular immunity in abortion results in a rejection of the embryo.TH-1 type cells induced by the infection may cross the interface of the fetus or may produce trophoblasticinflammatory cytokines[5].

The development of these cytokines is partially regulated by genetic regulation and the level of production of cytokines has been found to be associated with genetic polymorphisms, particularly TNF alpha and IFN gamma[6]. There is a relation between TNF-a gene polymorphism and abortion risk[7]. Mycotic abortion (fungal abortion, mycotic placentitis), caused by many fungi, is a cosmopolitan, contagious, intermittent, animal, particularly sheep, infection of the genital tract;moreover, Magee and Cox[8] found that passive

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serum transfer from the Formalin-killed spherules (FKS) mice vaccine did not protect the recipients; however, Raghupathy[9] who also noticed that neither serum nor immune B-cells transferred protection against mice attack. However, the role of a coccidioidomycosis humoral-immune response remains unclear[10].

In this study, real-time polymerase chain reaction (RT-PCR) detected the expression of TNF-α DNA in mycotic aborted sheep for Placenta; thus, revealing the link between aberrant TNF-α DNA expression and abortion. It is the first study that showed that mycotic aborted animals had increased expression of alpha TNF. In fact, changed expression of those inflammatory molecules can contribute to the pathophysiology of abortion.

Materials and Method

Fifteen samples of the mycotic of the aborted placenta, which was collected from aborted ewes, were collected from various areas of Al-Najaf City, and fresh placental tissue was subjected to complete DNA extracted using Genomic DNA Mini Extraction Kit (KAPA BIOSYSTEM, USA).

These samples were taken from November 2018 to November 2019, following instructions from the manufacturer RT-PCR for the molecular analysis of the genes TNF-alpha. Fresh placental tissue was subjected to complete DNA extraction using the Genomic DNA Mini Extraction Kit (KAPA BIOSYSTEM, USA), following the manufacturer instructions.

Quantitative Real-time PCR: Real-time PCR was made using TNF alpha primers (forward GAA TAC CTG GAC TAT GCC GA, reverse CC TCA CTT CCC TAC ATC CCT) according to the manufacturer's protocol (Applied Biosystem).

Quantitative RT-PCR reactions were conducted in 20 µl containing 10 µl KAPA SYBER* (KR0393_S – v2.17) FAST qPCR Master Mix(2X), 0.4 µl forward Primer(10 µM), 0.4 µl Reverse Primer(10 µM), 0.4 d UTP (10 µM), 0.4 ROX High/Low, 0.4 µl KAPA RT Mix (50x), and 3 µl deionized water DNA template up to 20 µl. The cycling conditions were as follows for all genes: 10 min at 95 ºC, 40 cycles of 30 seconds at 95 ºC,40 cycles of 30 seconds at 95 ºC,1 min at 55 ºC and 1 min at 72 ºC followed by a melt curve starting at 65 ºC rising to 94 ºCat 0.3 per-second**.**

Statistical Analysis: The differences between the means were analyzed with Student's t-test for unpaired samples; the *P-value <0.05* was considered to be significant the differences between. Analysis of the aborted and normal expressions of TNF alpha placental samples were performed using SPSS v.19.0 (SPSS Inc., Chicago, USA).

Results

The placenta immune response was characterized by an analysis of the aborted TNF-α andnormal placecentomes of delivery, Table(1). All the cytokines showed substantial increases compared to the animals given one. Those results were analyzed when aborted animals did not show any differences with the control sheep;thus, the interpretation was identical regardless of the duration of the abortion. Similarly, the increase in TNF-α transcription among the aborted groups was similar.

Table 1: Comparison of cytokine expression between two groups

	Mean	N	Std. Deviation	Std. Error
Normal	17.5200	15	0.76278	0.31141
Aborted	22.1067	15	3.05502	1.24721

TNF-α DNA levels expressed in Placenta differed considerably between the two groups (P<0.05) respectively. The number of cases showing over-expression of TNF alpha in the aborted placenta was 15 of 20 (80 %), which was statistically important from controls.

In placental aborted research median alpha, TNF fold transformation was found to be 22.1067 Relative to the controls which were 17.5200.

Discussion

The inflammatory response in abortion which is necessary for anordinary pregnancy is sought to be altered. Nonetheless, a motherly immune response is a primary determinant of success or failure in pregnancy[11]. However, the inflammation of mediated pregnancy is a well-studied phenomenon in rodents, which is used as a basis for spontaneous pregnancy loss. Further, Aberrant inflammatory and environment are related to uteroplacental perfusion dysfunction, the incidence of thrombotic events, and placental and fetal hypoxia[12].

Several studies on the role of TNF polymorphisms show the impact of the various alleles on *in vitro* and *in vivo* production of TNF levels[12]. Nevertheless, a recent study showed that not only polymorphisms within the TNF cluster are necessary to regulate the production of TNF but also receptors (TNF R)[13]. This result suggested that research into polymorphisms within the TNF cluster and TNF receptors will be important in understanding the role of TNF regulation in a given disease.

A substitution of (G) to (A) in role-308 in the tumour necrosis factor-alpha (TNF-alpha) gene promoter increases the transcription of TNF-alpha *in vitro* by around 6 to 9 folds[13]. Paracoccidioidomycosis (PCM) has associated polymorphisms in other genes, such as cytokines, which play a role in the immune response,which involve polymorphisms of IL-10(1082 G/A) and TNF-α (-308 G/A) in PCM patients It has been found that while homozygous, the IL-10-1082 G allele may be correlated with an increased risk of disease contracture[14].

Placental cytokines such as IL6, TNF α, and TGF β have been reported to play roles in necrotic and aponecrotictrophoblast cell death by influencing caspase and endothelial cell activation activities.IL6, IL1, and TNF α are thought to play important roles in early pregnancy and also to be elevated in inflammatory states[15]. High levels of pro-inflammatory molecules such as TNF a, IFN-gamma, IL-6andIL-10 and inflammatory leukocytes (macrophages, neutrophils, lymphocytes) were found in women with recurrent pregnancy loss (RPL) compared to women with normal pregnancy[16].

Baud and Karin[17], elevated levels of pro-inflammatory cytokine in the macrophage triggered by IFN-gamma and TNF-alpha.IFN γ was formed from trophoblasts and endometrial mucosal cells at moderate levels. It's consistent with other findings from the mouse study[18]. Since its ability to control immunologically important transcription, this is a type II interferon and controller of a wide variety of cellular processes. Therefore, this study hypothesized that maternal overexpression of the TNF alpha gene may increase the recruitment of inflammatory leucocytes in the maternal-fetal interface,resulting in uteroplacental perfusion deficiency, development of thrombotic events and placental hypoxia, finally abortion of embryos. This study also examined the altered expression of TNF alpha in 15 aborted placenta versus 15 normal placenta, subjects as controls. TNF alpha gene has been evaluated using quantitative real-PCR to assess if the pattern of differential expression of such transcript analysis has been performed.

The level of TNF alpha protein in the placental tissue of the aborted ewes has increased significantly ($p < 0.05$) compared to controls. Thus, this is the first study that predicted the role of the inflammatory molecules TNF alpha. The research opens up a new perspective to understanding the role of TNF alpha in pregnancy maintenance and outcome. In this context, Th2 or Th3 cytokines, such asIL-4, IL-10or TGF-b, would promote pregnancy survival, while the development of excessive pro-inflammatory cytokines (i.e., TNF-a Or IFN g) would assess fetal rejection[19].

The TNF-alpha is primarily expressed by mononucleomacrophage, CD4 + Th1, cell NK, etc. Yet apart from these immune cells, certain reproductive tissues can also express this cytokine[13]. It was observed that the expression of TNF-α DNA decreased significantly in normal delivery compared to that of abortion, which was consistent with Kirwan *et al.*[14] findings. Monzón-Bordonaba *et al.*[22]. reported that lower TNF-α concentrations could improve pregnant women's energy metabolism and their embryo development,increase synthetic progestagen and choriogonadotropin levels, and stimulate trophoblast to generate urokinase-type plasminogen activator (uPA),It promotes the deterioration of decidual cell ectomatrix and placenta implantation and eventually plays a role in pregnancy sustainability.

Chaouat *et al.*[23]. found that higher TNF-α concentrations can lead to abortion by the promotion of trophic cell apoptosis, the elevation of synthetic PG E2, excitation of uterine smooth muscle,stimulation of Th1 type of immunological reaction, rejection of embryonic tissue, coagulative system activation leading to placenta trophic vessel thrombosis. Thus, the proportion of CD4 + T cells in the peripheral blood of early pregnant women had decreased significantly, presumably as a result of physiological changes in several hormones during pregnancy contributing to certain changes in the maternal immune system and ensuring the development of relatively lower levels of TNF-α in pregnant maternity.

A study showed that abortion was related to an irregular rise in the TNF-α serum protein, which the expression of TNF-α increased significantly locally in

different types of cells at the maternal-fetal interface during a spontaneous abortion, and 94% of these TNF-α were located in the mother's peripheral blood cycles[24]. Furthermore, Zenclussen *et al.*[25] found that P-selectin within the vascular wall of decidua is significantly increased in patients with normal spontaneous abortion,and, in combination with P-selectin ligand on the surface of TNF-α-expressing Th1 cells, local migration of Th1 cells from the peripheral blood to the maternal/fetal interface results.

Chaouat *et al.*[26]reported that PBMCs were accumulated locally through a series of complicated mechanisms at the maternal-fetal interface and secreted a larger amount of TNF-α in the form of paracrine or autocrine, possibly resulting in abortion.Although the causal-effect relationship between abnormal expression of TNF-α and abortion is not yet clear, abortion may be correlated with TNF-α gene polymorphisms[27].A study has recently found, from the immunogenetic point of view, that the gene polymorphism occurred in women with a tendency to abortion, cytokine modification may typically be affected by many factors during a spontaneous abortion[28]. Nevertheless, the above study raised a train of new thoughts, the exact mechanisms of TNF-α leading to abortion are not completely understood,and whether the initiator or the successor case is an irregular expression has not been confirmed, requiring more research on many aspects of gene polymorphism, gene transcription, and expression of TNF-α and INF gamma receptors. Moreover, a recent study reported that a significant link between recurrent pregnancy loss and polymorphisms were studied. This research confirmed that TNF-α polymorphisms might suscept factor of recurrent pregnancy loss cases. Therefore, this study concluded that TNF-α polymorphisms were the possible genetic problem of pregnancy loss[29].

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

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